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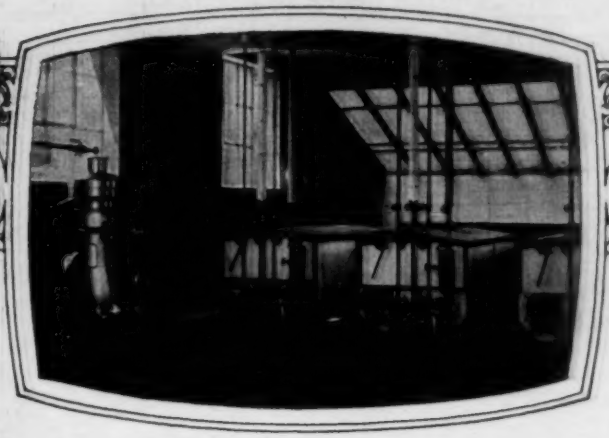

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(a) The general sanitary condition of the barns.
(b) Presence or absence of seeder cows in the herd.
(c) The methods of handling the milking machine.

¶ Since conditions (a) and (b) exist whether the milking is done by hand or by machine, and have been previously discussed, I will discuss here only milking machines.

¶ Observations by those studying this question show that lack of care on the following points is responsible for any high count milk which may be attributable *directly* to the *machine* and not to seeder cows, or other extraneous conditions:

(1) Permitting the machine to stand for considerable time after use before the cold water rinse.
(2) Insufficient water used in the rinse.
(3) Neglect to use a hot washing powder solution immediately following the cold rinse, or not hot enough.
(4) Using unreliable sterilizing solutions following the hot washing powder solution, and using improperly.
(5) Failure to maintain the soak solution (used between the milking periods) at standard strength.
(6) Failure to provide sufficient soak solution to completely cover cups and tubes, and to place properly so as to avoid air pockets which prevent complete contact.
(7) Failure to provide a pail of fresh sterilizing solution to dip cups in after milking each cow, to prevent carrying bacteria from an infected cow to the next, and into the composite milk of all.

Other factors *not attributable* to the machine,
but which cause poor results, are

Failure to disinfect the cows' udders and flanks before milking, with a cloth wrung in a proper disinfectant.
Failure to properly sterilize cans, buckets, strainers and all other equipment making contact with the milk.
Failure to provide prompt cooling of the milk to a sufficiently low temperature.
Failure to test frequently the milk of individual cows and to keep separately the milk of seeder cows which otherwise will contaminate the entire composite milk of the herd.

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STUDIES ON BUTTER SALTS*

O. F. HUNZIKER, W. A. CORDES, AND B. H. NISSEN

Blue Valley Creamery Company, Research Laboratory, Chicago, Illinois

PURPOSE OF INVESTIGATION

The salt in butter is a factor in commercial butter manufacture which has been held responsible, in many instances, for diverse butter defects, such as defects in flavor, body, texture, and color. Instances have also come to our attention of creameries claiming to be able to incorporate more salt and to secure a larger overrun with certain brands of salts than with others.

Bitterness is one of the principal flavor defects that has been ascribed to the influence of salt and this is commonly associated in the practical mind with the presence of impurities in the salt. Magnesium and calcium chlorides, for instance, have a bitter character and salt manufacturers have endeavored to produce butter salts that are as free as possible from these and other chemical impurities.

The question of ease of salt incorporation and the presence or absence of free moisture in butter have led to various claims regarding the influence of individual brands of salt. These questions, together with the usual difficulties in maintaining a satisfactory overrun and in avoiding grittiness in butter, are confronting the practical buttermaker with real problems.

The color of butter is known to be influenced by the salt. The appearance of mottles, streaks, and waves, has suggested the possibility of a relation between unevenness of color and type of salt used.

The purpose of the present investigation, therefore, was to determine if, and to what extent, the type and brand of butter salt is a factor in those problems of the buttermaker that have to do with the control of the overrun and with guarding against certain butter defects.

* Received for publication April 20, 1928.

SCOPE OF INVESTIGATION

Ten well known butter salts were included in this study. They were obtained in standard barrels taken from commercial stock. These salts are listed in this report according to number from 1 to 10. Three brands were flake salts and the remaining 7 were cube salts. The flake salts are represented by the numbers 1, 2, and 3. The remaining numbers refer to cube salts. Each salt was studied in accordance with the following outline:

Physical, chemical, and bacteriological analyses
Effect of salt on flavor, body, texture, and color of butter
Effect of impurities in salt on flavor of butter
Suitability for use in soaking parchment wrappers and liners

SAMPLING FOR EXAMINATION AND ANALYSIS

All samples for physical and chemical analysis were taken into dry Mason jars from near the center of the barrel after about 50 pounds of the salt had been removed. Samples for bacteriological examination were taken into sterile 4-ounce bottles.

PHYSICAL ANALYSIS

Condition of salt in barrel. Brands 4, 5, 6, and 8 were profusely caked, necessitating the use of a pick to loosen before removing with scoop. Brands no. 2, 3, 7, 9, and 10 showed slight caking into loose masses and lumps, but these broke down easily giving no handling difficulty. Brands no. 2 and 3 appeared damp and had little flowing tendency, while the other salts appeared dry and loose, and could be poured out like sugar.

Color of dry salts. Portions of the salt were placed on sheets of black paper and arranged in the order of their relative whiteness. Comparison was facilitated by pressing glass plates down on the piles of salt. The colors ranged from pure snow white to cream color. Nos. 3, 2, 10, 7, and 5, in the order given ranged from pure white to the first shade of cream. Nos. 1, 4, 9, 6, and 8, in the order given, showed increasingly marked creamy color.

Bulkiness or relative density. The bulkiness of the salts was determined, and is here expressed, in terms of relative density.

This was done by comparing the weight of a given volume of each salt with the weight of the same volume of water. For this purpose a 200 cc. assay flask was used. This flask was packed by uniform jarring against a solid surface. Checks within one gram were readily obtained between duplicates. The relative densities of the dry salt were as follows, using the density of water as 1.

Flake salts (nos. 1 to 3)..... 0.82–0.90 (average 0.86)
Cube salts (nos. 4 to 10)..... 1.23–1.28 (average 1.26)

The above figures show that the flake salts are of the lowest relative density, their weight being less than that of water. The

TABLE 1

Percentages of salt passing through screens of various mesh

SALT NUMBER	MESHES TO THE INCH						
	Over 20	20	30	40	60	80	100
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	0.2	15.6	8.2	40.7	20.7	8.0	6.6
2	0.2	16.2	9.1	34.0	19.5	9.6	11.4
3	0.3	19.2	9.7	49.4	16.9	3.1	0.4
4	Wood splinters	0.8	0.8	52.8	38.2	4.0	2.1
5	Few grains	0.6	0.6	63.0	28.8	3.8	2.4
6	None	0.6	0.6	46.9	43.6	4.8	2.5
7	None	1.0	1.3	55.4	30.6	5.5	5.5
8	None	1.0	1.1	49.0	38.8	4.5	4.8
9	None	1.3	1.0	39.2	44.6	7.2	6.3
10	None	0.6	0.4	48.8	46.0	3.0	0.5

cube salts are considerably heavier than water per unit of volume. Thus, the flake salts are lighter and more bulky than the cube salts. When packing the usual standard weight of 280 pounds of salt into the commercial package, the barrel for the cube salt need be only two-thirds as large as that for the flake salt.

Size of salt grains. Fifty-gram samples of salt were placed in the topmost of a series of sieves, of 20, 30, 40, 60, 80, and 100 mesh. The sieves were then shaken for five minutes, or until less than 0.3 gram additional salt passed through any screen in one minute. The percentages of salt passing through each sieve were determined, and are listed in table 1.

The above figures indicate that the great majority of all the salts passed through 40 and 60 mesh screens. The flake salts, however, proved considerably coarser than the cube salts and their range in crystal size was wider, while the cube salts show great uniformity in size of crystal.

Microscopic examination of salts. The salt grains were also studied under the microscope, using a magnification of 75×, with the salts immersed in an oil. By applying slight pressure to one edge of the cover glass the crystals could be made to rotate in the oil thus permitting examination of all sides of the crystals. Measurements of the crystals were made by means of an eye-piece micrometer. Table 2 gives the predominating size and mesh, as estimated from these measurements.

TABLE 2

Size and shape of crystals

SALT NUMBER	TYPES OF CRYSTALS	SIZE OF INDIVIDUAL CRYSTALS	MESH OF CRYSTALS (ESTIMATED)
		mm.	
1	Flakes	0.16–1.6	50–150
2	Flakes	0.1 –0.6	25–200
3	Flakes	0.15–0.90	25–150
4	Cubes	0.48	40–60
5	Cubes	0.13–0.24	40–100
6	Cubes	0.1 –0.4	50
7	Cubes	0.16–0.43	40–100
8	Cubes	0.15–0.30	40–150
9	Cubes	0.08–0.32	50–100
10	Cubes	0.20	60

TABLE 3

Turbidity and color of saturated water solution

SALT NUMBER	TURBIDITY	COLOR OF SOLUTION	SALT NUMBER	TURBIDITY	COLOR OF SOLUTION
1	108	Gray, turbid	6	40	Deep brown, turbid
2	5	Colorless, clear	7	8	Slight brown, clear
3	5	Colorless, clear	8	12	Slight brown, turbid
4	60	Brown, turbid	9	10	Slight brown, slightly turbid
5	3	Colorless, clear	10	5	Colorless, clear

Turbidity and color of salt solution. The turbidity was determined by comparing saturated solutions of the salt with turbidity standards prepared by mixing one gram of 200 mesh Fuller's earth in one liter of distilled water (A. P. H. A. Standard Methods of Water Analysis, 1925). These observations were made in 12-ounce bottles and also by the use of Nessler tubes.

Table 3 shows a considerable variation in the turbidity and color of the brines prepared from these salts. This character of the salt is not apparent where the use of the salt is confined to incorporation in butter, but when used in solution for soaking parchment wrappers and liners this turbidity becomes an objectionable property,—in fact, the turbidity of the brine from

TABLE 4

Sediment and insoluble matter

SALT NUMBER	APPEARANCE OF SEDIMENT	AMOUNT OF INSOLUBLE MATTER	SALT NUMBER	APPEARANCE OF SEDIMENT	AMOUNT OF INSOLUBLE MATTER
		per cent			*per cent*
1	Gray brown	0.031	6	Dark brown	0.024
2	Light brown	0.007	7	Gray	0.009
3	Gray	0.004	8	Very dark brown	0.011
4	Dark brown and specked	0.032	9	Light brown	0.006
5	Light gray	0.003	10	Black	0.005

salts no. 1, 4, and 6, was so marked as to render these salts unfit for this purpose.

Insoluble matter. Saturated solutions of the various salts were passed through previously dried and weighed filter papers. These were again dried and weighed, giving the weight of insoluble matter in the dry salt. The appearance of the sediment and the per cent of insoluble matter are given in table 4.

The sediment was treated with 2 cc. concentrated hydrochloric acid. The solutions in all cases became perfectly clear. This suggests that the sediment was mainly salts of calcium and iron, probably largely calcium carbonate.

Moisture content. The moisture content of the salt has a direct bearing on its flowing property and caking tendency.

Ten gram samples of salt were dried at 115°C. until constant in weight. The results are shown in table 5.

Caking tendency. A small portion of each salt was left exposed to the air in a Petri dish for several days. All salts excepting nos. 7 and 9 showed considerable caking. These two salts still possessed their free-flowing property after such exposure to air. By referring to table 5, it will be noted that these two salts contained the least amount of moisture.

It was noted also that the addition to the dry salt of as little as 0.05 per cent water caused the salt to lose its free-flowing property and to appear damp. The addition of from 0.1 to 0.5 per cent water produced an even greater change in the same direction. When these moistened salts were exposed to the air they caked

TABLE 5

Moisture content of salts

SALT NUMBER	MOISTURE	SALT NUMBER	MOISTURE
	per cent		*per cent*
1	0.08–0.10	6	0.12 –0.12
2	0.10–0.10	7	0.005–0.01
3	0.15–0.13	8	0.14–0.14
4	0.07–0.07	9	0.01–0.01
5	0.14–0.13	10	0.03–0.03

very profusely. This suggests that freedom from caking requires as nearly complete absence from moisture as possible.

The addition of 2 per cent calcium sulphate or 3 per cent potassium chloride to pure salt did not increase the caking tendency. The addition of 1 per cent calcium chloride or 1 per cent magnesium chloride, because of their deliquescent properties, however, caused the salt to cake. In the case of magnesium chloride the salt became and remained damp, but it did not cake to the same extent as with calcium chloride. These results suggest that the presence, in abnormal amounts, of calcium or magnesium chloride in butter salt is objectionable because these impurities cause the salt to become damp, or to cake, or both.

Rate of solution. Solubility tests of each salt were made as follows: A definite amount of salt was added to a given amount

of water. The liquid was kept in constant agitation by means of a motor stirrer and maintained at a constant temperature during the entire test. Samples were removed at carefully timed intervals and the percentage of salt determined by gravimetric analysis.

Since sodium chloride goes into solution very rapidly, it was necessary to remove samples quickly and at short intervals. This was accomplished by the use of 25 cc. pipettes cut off at the lower part of the bulb. Over this opening were stretched two layers of fine-mesh silk with a cotton pad or sediment disc between them.

TABLE 6

Solubility rates of butter salts

Percentage of salt in solution at various time intervals

SECONDS	SALT NO. 1	SALT NO. 2	SALT NO. 3	SALT NO. 4	SALT NO. 5	SALT NO. 6	SALT NO. 7	SALT NO. 8	SALT NO. 9	SALT NO. 10
5	20.20	22.40	21.28	20.82	19.75	21.03	20.54	20.04	21.14	20.82
10	23.72	24.33	23.63	23.66	22.95	23.76	23.27	23.38	23.84	23.67
15	24.67	25.22	24.80	24.72	24.32	24.99	24.63	24.59	24.87	24.74
20	25.35	25.60	25.24	25.30	24.94	25.54	25.13	25.22	25.39	25.27
25	25.62	25.87	25.45	25.73	25.45	25.91	25.65	25.68	25.70	25.72
30	25.75	25.97	25.67	25.71	25.52	26.07	25.78	25.94	25.92	25.90
40	26.10	26.19	25.96	25.86	25.98	26.34	26.12	26.22	26.09	26.14
60	26.25	26.32	26.19	26.17	26.26	26.35	26.24	26.43	26.24	26.31
90	26.33	26.39	26.29	26.31	26.22	26.51	26.30	26.47	26.30	26.38
120	26.35	26.43	26.34	26.40	26.48	26.37	26.39	26.54	26.32	26.40
180	26.38	26.43	26.36	26.44	26.47	26.51	26.40	26.55	26.34	26.40
240	26.30	26.42	26.37	26.44	26.54	26.66	26.39	26.56	26.33	26.39

The container was immersed in a water thermostat at 55°F. and each salt was properly tempered before addition to the water. The solubility determinations were made by placing two liters of water adjusted to exactly 55°F. in the container. The salt (740 grams) was weighed into a scoop so constructed as to discharge the entire amount of salt into the 2 liters of water in one second of time or less. With the water in vigorous agitation the salt was added and samples amounting to approximately 20 cc. were removed at definite intervals. In order to standardize this part of the procedure, the time for taking each sample was limited to exactly three seconds. The pipette was emptied by

inverting it so as to prevent crystals adhering to the outside of the silk from being washed into the sample bottle. The per cent salt was determined immediately by evaporation of 10 gram portions. To prove that no crystals of salt passed through the above filter pipette, check determinations were made with a Mandler diatomaceous filter of the Berkefeld type. The results of these tests of solubility rates are given in table 6. These results represent averages of several trials with each brand of salt.

Table 6 indicates that the difference in rate of solution between the several salts is very slight and not sufficient to justify any preference for one brand of salt over another.

Solubility tests were also made by using glass tubes 43 inches in length and $\frac{1}{8}$ inch in internal diameter. The tubes were filled with water and 10 grams of the salt were discharged into each tube, allowing the salt crystals to settle through the water. A whitish turbidity was noted in the case of every salt but this disappeared quickly. This turbidity was due to air bubbles, either locked up in the salt or mechanically carried with the salt into the liquid. There was not much difference in the depth to which the salt crystals dropped before they dissolved, though there appeared to be a tendency for the flake salts (1, 2, and 3) to drop to a lower depth, a few crystals actually reaching the bottom of the tube.

This test cannot be considered a very dependable index of the rate of solution of the salt when used in butter. The concentration of the solution is not sufficient to match the concentration of the brine in butter and the fact that, as the crystals sink through the water they strike fresh water continuously, presents a condition entirely different from that existing in the salting of butter.

CHEMICAL ANALYSIS

Reaction of saturated brine to litmus paper. A saturated solution of the salt was prepared with boiled, distilled water. Each solution was tested with litmus paper with the following results:

Neutral: Salts 2, 3, 5, 6, 8, and 10
Alkaline: Salts 1, 4, 7, and 9

Methods of chemical analysis. Samples for chemical analysis were dried on Petri dishes in the electric oven at 115°C.

Phosphates. One hundred grams of the salt were dissolved in 300 cc. of water. Ten cubic centimeters of nitric acid were added and then concentrated ammonia until almost neutral to litmus paper. Next, 40 cc. of 4 per cent ammonium molybdate were added. The mixture was heated to about 60°C. and allowed to stand at room temperature for one hour. Absence of bright yellow color or yellow precipitate indicated absence of phosphates.

Iron oxide and alumina. One hundred grams of the salt were dissolved in distilled water to saturation and about 10 drops of concentrated nitric acid added. The solution was boiled for about one-half hour to oxidize the iron, then a slight excess (0.5 cc.) of ammonia was added, boiling was continued until the odor of ammonia disappeared. The solution was allowed to stand overnight, then filtered, washed with hot water until free of chlorides, dried, and ignited in a small platinum crucible. The results are reported as $Fe_2O_3 + Al_2O_3$.

For the following analyses 100 grams of the dried salt were dissolved in distilled water and made up to 500 cc. One hundred cubic centimeters of this solution represents 20 grams of salt.

Total lime. One hundred cubic centimeters of the above salt solution (representing 20 grams of salt) were freed of iron and filtered. The solution was then heated to boiling. Ten cubic centimeters of 10 per cent oxalic acid were added. A few drops of methyl orange were added and the solution was neutralized with concentrated ammonia, added very slowly. One cubic centimeter excess of ammonia was added and the solution allowed to stand for three hours in a warm place. It was then filtered and washed once with 10 cc. of 1 per cent ammonium oxalate. The filtrate and washings were saved for the magnesium determination. The precipitate on the paper and in the beaker was dissolved with hot dilute HCl, then diluted to 100 cc. and again precipitated as before; it was filtered after three hours standing and washed with 1 per cent ammonium oxalate until free of chlorides. The filter was then ignited in a platinum crucible and the precipitate weighed as CaO.

Magnesium oxide. The washings and filtrate from the calcium determination were concentrated to 150 cc., 3 grams of diammonium phosphate and a few cubic centimeters of dilute HCl being added to clear the solution. The solution was allowed to cool and was then made alkaline with ammonia; 2 cc. excess NH_4OH were added. The mixture was allowed to stand overnight. It was then filtered, washed with a little dilute (1:10) ammonia, dissolved in HCl (1:1) and precipitated again and filtered as above. It was washed with ammonia (1:10) until free of chlorides, the test drop being neutralized with dilute HNO_3 prior to addition of $AgNO_3$. The filter was ignited in a platinum crucible, weighed as magnesium pyrophosphate and reported as magnesium oxide.

Sulphur trioxide. To 100 cc. of the original salt solution (representing 20 grams of salt) 5 cc. of dilute HCl were added and the solution heated to boiling. Five cubic centimeters of a 10 per cent $BaCl_2$ solution diluted to 100 cc. were then added gradually while hot, allowing to stand overnight. It was then heated and filtered while hot and washed with hot water until free of chlorides. The filter was ignited in a platinum crucible and weighed as $BaSO_4$, from which the SO_3 content was calculated.

Barium salts. No barium salts can be present in these salts since soluble sulphates were present in all.

Sodium chloride by gravimetric determination of chlorides. Fifty cubic centimeters (10 grams salt) of the original solution were diluted to 500 cc. Twenty-five cubic centimeters of this solution, representing 0.5 gram of NaCl, were pipetted out and diluted to about 250 cc. in a 400 cc. beaker and 5 cc. of dilute nitric acid added. Twenty cubic centimeters of 10 per cent $AgNO_3$ were then added slowly while stirring vigorously. It was allowed to stand in a dark place overnight, and then filtered on a Gooch crucible containing a layer approximately 0.5 cm. thick of long-fibre, acid washed asbestos, thoroughly washed with water prior to each use. The silver chloride precipitate was then washed with water containing a few drops of a solution of silver nitrate until only a faint test for nitrates was noted. Then it was finally washed with about 10 cc. of boiling hot water, and

TABLE 7

Chemical analyses of butter salts

Percentage composition

SALT NUMBER	SODIUM CHLORIDE		CALCIUM SULPHATE	CALCIUM CARBONATE	CALCIUM CHLORIDE	MAGNESIUM SULPHATE	MAGNESIUM CARBONATE	MAGNESIUM CHLORIDE	SODIUM SULPHATE	$Fe_2O_3 + Al_2O_3$	INSOLUBLE MATTER	PHOSPHATES	BARIUM SALTS	MOISTURE
	By direct analysis	By difference												
1	99.19	99.76	0.073			1.38				0.002	0.031	0.00	0.00	0.08
2	98.92	99.58	0.302		0.078			0.025		0.002	0.008	0.00	0.00	0.105
3	99.48	99.88	0.027		0.048			0.041		0.003	0.004	0.00	0.00	0.140
4	99.11	99.19	0.699	0.046			0.027			0.008	0.031	0.00	0.00	0.070
5	99.08	99.18	0.756		0.059			0.006		0.001	0.003	0.00	0.00	0.135
6	98.34	98.59	1.225		0.116			0.037		0.006	0.027	0.00	0.00	0.125
7	99.69	99.90	0.010			0.010			0.071	0.003	0.009	0.00	0.00	0.005
8	98.67	98.90	0.996		0.060			0.026		0.002	0.012	0.00	0.00	0.140
9	99.57	99.94	0.038	0.007			0.011			0.001	0.006	0.00	0.00	0.010
10	99.39	99.77	0.197		0.022			0.013		0.003	0.005	0.00	0.00	0.032

Salts 3 and 9 were analyzed for KCl and found to contain less than 0.06 and 0.02 per cent respectively.

dried at a temperature of 105°C. overnight. The residue was placed in a large platinum crucible and heated gently with a Bunsen flame until the edges of the AgCl just began to fuse. It was then cooled and weighed as AgCl. From the figure thus obtained must be subtracted the silver chloride originating from chlorides other than that of sodium. Sodium chloride is so reported in the analyses.

Tabulated results of chemical analyses of dairy salts. The results listed in table 7 are on a moisture-free basis. In each case the method used was previously checked against mixtures of known

TABLE 8

Bacteriological analyses of salts

SALT NUMBER	BACTERIAL COLONIES		MOLD COLONIES		TOTAL COLONIES	
	4 gram total	Per gram salt	4 gram total	Per gram salt	4 gram total	Per gram salt
1	6	1–2	0	0	6	1–2
2	8	2	1	<1	9	2–3
3	3	<1	0	0	3	<1
4	23	5–6	2	<1	25	6–7
5	4	1	4*	1–2	9	2–3
6	5	1–2	0	0	5	1–2
7	0	0	2	<1	2	<1
8	6	1–2	1	<1	7	1–2
9	3	<1	1	<1	4	1
10	9	2–3	4	1	13	3–4
Average.....	6–7	1–2	1–2	<1	8–9	2–3

* 1 yeast.

chemical composition. Sodium chloride is reported both by direct analysis and by difference, i.e., by subtracting impurities from 100.

BACTERIOLOGICAL ANALYSIS

Methods. The various salts were examined for total number of bacteria and molds. This was done by plating on beef infusion agar. One gram of salt was weighed directly into a sterile Petri dish. Five cubic centimeters of sterile distilled water were added to dissolve the salt before pouring the agar. Incubation was at

21°C. for four days, followed by two days at 37°C. Two examinations of each salt were made from different parts of the barrel.

Preliminary plating was also done on whey agar with 1 cc. of a 1 per cent solution of lactic acid per plate to give favorable conditions for mold growth. It was found, however, that beef infusion agar gave fully as positive results and consequently whey agar counts were discontinued.

A 10 per cent solution of each salt was also made in sterile distilled water and 1 cc. of this solution was added to the Petri dish in order to allow a large dilution and to avoid any inhibitory action which might be exerted in the case of the greater salt concentration. This method, however, gave no colonies at all on some plates and only one colony on others, indicating that the plating of one gram of salt in each dish without dilution was the only method to be relied upon for securing the true germ content of the salt. The results are given in table 8.

The results in table 8 indicate that all of the salts studied are low in germ life and none of them, if handled properly and kept in the condition in which they exist in their original container, the sealed barrel, would be a source of contamination of any consequence in the manufacture of butter.

EFFECT OF SALT ON FLAVOR, BODY, AND COLOR OF BUTTER

Method. A sufficient quantity of unsalted butter taken from a factory churning was used for each entire salt series. Thirty pounds of this butter were worked up with each salt in a small combined churn and worker. The butter was first worked in water in an effort to have all lots of butter in the same condition as to body, texture, and temperature before and at the time the salt was added. The amount and temperature of the water used and the number of revolutions worked were the same for all lots.

After draining the water out of the churn the same amount of salt was added to the butter in each case (aiming at 3 per cent salt in the finished butter) and the same amount of water was used to wet the salt in the trench. After closing the trench the butter was worked the same length of time for each salt. It was

then packed in 30 pound tubes and placed in storage at 40°F. until scored. The churn was washed out between consecutive lots of butter to remove all remnants of brine. Two entire series of this experiment were made. The tubs were all numbered for scoring and no judge had any knowledge of the identity of any of the tubs. The butter was examined by three judges for flavor, body, color, and salt. Particular attention was paid to the detection of bitterness, brininess, and the presence of undissolved salt as "grit."

Results. Careful examination and re-examination of each tub of butter by the three judges failed to reveal any consistent or significant differences. Comparisons of the various lots of butter for bitter and briny flavors gave convincing evidence that the brand of salt had no effect. They further showed very clearly that it is the method employed in the working of the butter and in the incorporation of the salt that is of primary importance and that controls the presence or absence of the objectionable briny, bitter flavor in butter. No difference in the shade or uniformity of color was detected among the several churnings.

The presence of "grit" due to undissolved salt was not restricted to any particular brands or types of salts, and consistent results were not always secured with the same salt. This finding has been confirmed under practical conditions in plants actually having difficulties in the complete solution and incorporation of salt in their butter. A change in the type of salt crystals from flakes to cubes produced no appreciable difference in the degree of grittiness and when the conditions of incorporating the salt and working the butter were changed, the grittiness disappeared with both types of salts.

Comparisons between a number of the salts were also made on a large scale under commercial operation in the Chicago factory. Separate churnings from the same cream out of the same vat were worked into salted butter, a different salt being used for each churning. Thus, here too, all interfering factors influencing the flavor were eliminated, facilitating detection of flavor differences due to salt. Careful examination of this commercial butter failed completely to show any differences in flavor, body, texture, and color.

On the basis of these findings we are forced to conclude that, as far as the brands of salts included in this experiment are concerned, the particular brand had no effect on the quality of the butter.

EFFECT OF IMPURITIES IN SALT ON BUTTER

Method. In this experiment such chemical impurities as are most likely to be encountered in salt were mixed with different portions of a pure salt before adding the salt to the butter. The impurities used were potassium chloride, calcium chloride, magnesium chloride, calcium sulphate. Potassium chloride possesses a penetrating, pungent character, and magnesium and calcium chlorides are bitter and biting to the tongue. It would be expected that their presence in butter salt in abnormal amounts would tend to impart a bitter flavor to the butter. Calcium and magnesium chlorides are further objectionable because of their deliquescent property. Their presence causes salt to absorb moisture from the air and to cake (see Physical Analysis). The amount of impurity mixed with the salt was greatly in excess of that present in any of the ten salts used in this investigation. Only one impurity was added to a salt for a single test. Each set of tests also contained a check churning of the same butter in the manufacture of which the pure salt only was used. The mixed impure salt contained the following percentages of impurities respectively:

	per cent
Potassium chloride	3
Calcium chloride	1
Magnesium chloride	1
Calcium sulphate	2

The salts prepared with the above impurities and the pure salt with no added impurity were worked into different lots of butter from the same churning. In each case the butter was so salted and worked as to secure a final moisture and salt content of as nearly 16 and 3 per cent respectively as possible. Four sets of comparisons were made, each consisting of one pure and two impure salts. Three judges examined each set of samples. The

judges were ignorant of the salt which the different samples represented.

Results. In spite of repeated scoring no consistent differences in flavor between the various tubs of butter could be established. These results substantiate and explain the absence of any visible difference in the flavor of the butter salted with the different brands of salt as discussed under "Effect of salt on flavor, body, and color of the butter." If the addition of these comparatively large percentages of impurities to the salt fails to have a sufficient influence on the quality of the butter to be noticeable to taste, it may be readily understood why different brands of salt in which the several chemical impurities are present in much smaller percentages fail to produce any differences in the quality of butter detectable by the sense of taste.

These findings suggest that the importance of slight differences in the chemical composition of butter salts has been somewhat exaggerated. It should be understood, however, that in these experiments the moisture was well incorporated and there was no indication of leakiness. In the case of very leaky butter, the free brine would undoubtedly give the palate a better opportunity to detect the bitterness which accompanies these impurities. Because of this possibility we must conclude that the use of butter salts containing excessive amounts of chemical impurities may jeopardize the flavor of the butter and is, therefore, to be avoided.

SUITABILITY OF SALTS FOR BRINE TREATMENT OF WRAPPERS AND LINERS

In the preparation of the butter package it is common practice to soak the parchment wrappers and liners in saturated brine for the purpose of preventing or retarding mold growth on the surface of the butter. In order to have this brine sterile it is usually heated to the boiling point by injecting live steam. In the case of some salts this heating of the brine causes the formation of a heavy precipitate, often of marked coloration, and the collection of a sediment, all of which is objectionable.

Tests made with the different salts gave varying results. The color, turbidity, and amount of sediment in the brine are given in table 9.

TABLE 9

Comparison of brines for treatment of parchment

SALT NUMBER	APPEARANCE OF BRINE	TURBIDITY OF BRINE	SEDIMENT IN 1 QUART OF BRINE
			mgm.
5	Good	5	10.4
2	Good	6	4.8
3	Good	7	3.0
7	Fair	15	10.2
10	Fair	11	4.5
4	Poor	80	53.5
1	Poor	160	
6	Poor	60	44.3
8	Poor	30	12.3
9	Poor	20	11.7

The first three salts listed in the above table gave a relatively clear brine, and salt no. 3 was judged best. The salts marked "poor" produced brines which could not be used for soaking parchment wrappers. Brines no. 1, 4, 6, and 8, were very turbid and brown in color. Salt no. 9 produced brine of intense yellowish brown color with a large amount of brown sediment. It cleared somewhat, however, upon standing overnight and at that time it was not as turbid as numbers 6 and 8. It is obvious that only such salts should be used for brine treatment of parchments as will produce a brine that does not place any deposit or sediment on the wrapper which lies next to the butter.

SUMMARY

1. Ten leading butter salts, representing three flake salts and seven cube salts, were studied with reference to their physical, chemical, and bacterial properties as related to their effect on flavor, body, texture, and color of butter.

2. Some of the salts were badly caked in the barrel while others were free-flowing. The most freely flowing salts were those that showed the greatest freedom from chemical impurities and that

were lowest in moisture content. Numerous tests showed that the drier the salt the less its tendency to lump. As the moisture content of the salt increases the salts lose their free-flowing property and cake profusely. For similar reasons the presence in salt of calcium chloride and magnesium chloride, which are highly deliquescent, diminishes the free-flowing property of the salt and causes it to lump.

3. The flake salts were found to be considerably more bulky than the cube salts. Taking the weight of a given volume of water as one, the weight of the flake salts averaged 0.86 and of the cube salts 1.26. With the weight of the commercial package of salt standardized to 280 pounds per barrel, the barrel for cube salt need be only two-thirds as large as the barrel for flake salt.

4. Several of the salts contained foreign matter in sufficient amounts to produce a very turbid brine and dirty color when dissolved in water. Such salts are objectionable, particularly when intended for use in the preparation of brine for the treatment of parchment liners, circles, and wrappers.

5. The salts in the original containers were found to be bacteriologically clean. The bacterial counts ranged from less than one colony per gram of salt to seven colonies, averaging from 2 to 3 colonies for all salts. Unless contaminated in the creamery after the barrel is opened, due to improper storing or handling, these salts may be considered entirely negative as a possible source of bacterial contamination of butter.

6. The great majority of the crystals in each salt passed through 40 and 60 mesh screens. The flake salt showed a considerably coarser grain and a wider range of crystal size than the cube salt. Thus, of the flake salts about 60 per cent of the crystals passed through 40 and 60 mesh screens while 27 per cent required a coarser screen. Of the cube salts, approximately 90 per cent of the crystals passed through the 40 and 60 mesh screens while only 1.7 per cent required a coarser screen.

7. The ten salts were very similar in their rates of solution. During the first twenty seconds there was a tendency for the flake salts to show slightly greater rapidity of solution than the

cube salts but at the end of twenty-five seconds the amount of salt dissolved averaged the same for both types of crystals. At the end of two minutes the solution of each salt was complete. After the first five seconds each liquid contained 19.75 per cent or more of salt in solution. This salt concentration is fully equal to the strength of the brine in butter containing 3 per cent salt. This rapidity of solution of each of the ten salts suggests that such minute differences in solubility rate as were observed between individual brands and between the two types of salt are too slight to be of any significance from the standpoint of preference for any one salt in butter manufacture.

8. The percentage of sodium chloride in the 10 salts, as determined by direct analysis, ranged from 98.34 to 99.69 per cent, averaging 99.14 per cent. The sodium chloride content as determined by difference ranged from 98.59 to 99.94 per cent. Phosphates and barium salts were entirely absent in all salts. The largest chemical impurity consisted of calcium sulphate ranging from 0.01 to 1.225 per cent. Small amounts of calcium and magnesium chlorides, magnesium and sodium sulphates, and calcium and magnesium carbonates, and traces of iron, were also present. The insoluble matter ranged from 0.003 to 0.031 per cent and the moisture from 0.005 to 0.14 per cent.

9. When working these salts into butter, both under experimental conditions and in commercial manufacture, no differences in flavor, body, texture, and color could be detected in the finished butter. Likewise, the addition to the pure salt of impurities in relatively large amounts, such as 2 per cent $CaSO_4$, 3 per cent KCl, 1 per cent $CaCl_2$, and 1 per cent $MgCl_2$, failed to have any noticeable effect on the flavor of the butter.

10. These findings suggest that, while chemical purity in butter salts is highly desirable, such small amounts of chemical impurities as are found in the above standard butter salts are incapable of impairing or changing the quality of butter.

DESTRUCTION OF BOTULINUM TOXIN BY MILK BACTERIA*

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Data have been published elsewhere, (Sherman, Stark, and Stark, (8)) which show a definite, though slight, destruction of botulinum toxin by intestinal bacteria. The intestinal bacteria studied are also commonly found in milk. The present paper reports work of a similar nature dealing with other types of milk bacteria in pure culture together with some observations on the action of a mixed flora.

The unique freedom of milk and milk products as agents in the dissemination of botulism is of great interest. Although fresh milk may ordinarily be used before bacterial growth has been sufficiently extensive to be dangerous from the standpoint of botulinum poisoning, such is not at all the case with cheese and certain other milk products. Indeed, from the standpoint of chemical composition, cheese would appear to be an excellent medium for the production of this toxin. The explanation which might appear valid is that the acidity of most types of cheese is sufficiently high to inhibit the growth of *Clostridium botulinum* but such an explanation would not hold for all types of cheese, and from some of the experimental results which have been published concerning the acid limits for growth of this organism, would indicate that a complete explanation cannot be founded entirely on the basis of acidity in the case of any type of cheese.

Jordan (5) puts the acid limit of growth of *Clostridium botulinum* at pH 4.9. It is well known that milk develops a lower pH than this after it is coagulated by acid in normal souring. This, however, would not account for the disposition of the toxin which might be produced while *Clostridium botulinum* is growing, before sufficient acid is produced to inhibit its

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action. Bronfenbrenner and Schlesinger (1) found that increasing the acidity to pH 4.0 caused botulinum toxin to become many times more toxic. Dozier (3) says, "The decline in numbers of viable organisms is followed by autolysis, which is probably the mechanism of toxin formation."

Nevin (7) records an instance in which three persons died of botulinum poisoning from eating home-made cottage cheese. She was able to isolate a type B strain of *Clostridium botulinum* from a sample of this cheese. In 1914, when these deaths occurred, but little information was available concerning this type of poisoning and "home-made cottage cheese" tells us little of the nature of the product from which the sample came.

Edmondson, Thom, and Giltner (4) inoculated three kinds of milk, designated by them as "sterilized," "laboratory pasteurized" and "commercial" (raw), with toxin-free spores of *Clostridium botulinum* and incubated them at various temperatures. Guinea pigs were fed this milk in order to test for the presence of toxin. They found that toxin was produced in the sterilized and laboratory pasteurized but not in the raw commercial milk. They say, "*B. botulinus* failed to produce toxin at any temperature in the low grade commercial product. No analysis of this result has been possible."

With slight changes, the experiment of Edmondson, Thom, and Giltner has been repeated by us. Only two incubation temperatures, 20° and 37°C. were employed. The samples of milk were inoculated into guinea pigs subcutaneously. Koser, Edmondson, and Giltner (6) found at least 500 times as much toxic material required when given by mouth to produce a similar effect as when injected intraperitoneally. In the first experiment, the results of which are recorded in table 1, the milks were lightly inoculated with detoxified spores of *Clostridium botulinum* and incubated for twenty-one days. At the end of this period guinea pigs were inoculated with 0.5 cc. amounts. In those samples in which noticeable proteolysis was absent filtration was difficult and in such cases the inocula were not filtered. Enough checks were run with the filtered and unfiltered product to convince us that the guinea pigs inoculated with unfiltered milk cultures, which

did not show proteolysis, did not die because of secondary infections caused by other bacteria which were present in the milk. The milk used in these experiments was of ordinary market quality.

All of the pigs in this experiment died from one to ten days after being inoculated, except one pig inoculated with raw milk which had been incubated at 20°C.

TABLE 1

The effect of bacteria on the accumulation of toxin in milk inoculated lightly with detoxified spores of Clostridium botulinum

VARIETY OF MILK	INCUBATION TEMPERATURE	TOXICITY TO GUINEA PIGS
	°C.	
Sterilized	37	Dead in 24 hours
Pasteurized	37	Dead in 8 days
Untreated	37	Dead in 2 days
Sterilized	20	Dead in 24 hours
Pasteurized	20	Dead in 10 days
Untreated	20	Survived

TABLE 2

The effect of bacteria on the accumulation of toxin in milk inoculated heavily with detoxified spores of Clostridium botulinum

VARIETY OF MILK	INCUBATION TEMPERATURE	TOXICITY TO GUINEA PIGS
	°C.	
Sterilized	37	Dead in 24 hours
Pasteurized	37	Dead in 2 days
Untreated	37	Dead in 10 days
Sterilized	20	Dead in 2 days
Pasteurized	20	Dead in 9 days
Untreated	20	Dead in 9 days

In repeating this experiment, the results of which are recorded in table 2, heavier inoculations were used, approximately 50,000 detoxified spores per cubic centimeter of milk. The incubation period was fifteen days. The amount injected into the test pigs was the same (0.5 cc.).

All of the guinea pigs given any one of the three kinds of milk,

incubated at either 20° or 37°C., were dead at the end of ten days. A possible interpretation of these results will be given later.

In order that we might have more definite information, the action of certain types of milk bacteria upon botulinum toxin was tested. Twenty-seven cubic centimeters of sterile meat infusion nutrient broth were placed into sterile test tubes. To each of these tubes of broth were added 3 cc. of botulinum toxin fluid. This toxic material was prepared by inoculating *Clostridium botulinum* into sterile milk and incubating under anaerobic conditions for seven days at 37°C. At the end of this incubation period the peptonized milk was filtered through a

TABLE 3

Action of milk bacteria upon botulinum toxin

	M.L.D. TOXIN INJECTED			
	1	2	4	8
Controls	+	+	+	+
Streptococcus lactis	−	−	−	+
Lactobacillus casei	−	−	−	+
Proteus vulgaris	−	−	+	+

+ = death of guinea pig with typical symptoms of botulism. − = survival of guinea pig.

Berkefeld filter to free it from organisms. This sterile toxic material was held for six months in a cold room having a temperature just above the freezing point. The toxicity of this material as determined just previous to its use in these experiments was approximately 100 M.L.D. per cubic centimeter. This would give us a toxin-broth mixture containing, at the beginning of the incubation period, approximately 10 M.L.D. per cubic centimeter. Table 3 shows the results of the action of certain milk bacteria upon botulinum toxin. In this table the unit amounts of toxin given are in terms of approximate M.L.D. values as determined on the control tubes after incubation.

After fifteen days' incubation at 37°C. and filtration, this toxin-broth mixture into which no organisms were placed was

found to contain about 1 M.L.D. of toxin per cubic centimeter. Dack (2) found a reduction of two times the original titer from ten days' incubation at 37°C. when using a toxin which had been stored for two months in the refrigerator.

The results from the action of these milk bacteria upon botulinum toxin show a very definite destruction of this toxin. Since it has been shown that certain prevalent milk bacteria have the power to destroy botulinum toxin, we are able to make some interpretations of the results obtained by Edmondson, Thom, and Giltner (4). Twenty degrees Centigrade is near the lower temperature limit for growth of *Clostridium botulinum*, while many of the bacteria common to milk grow well at this temperature. The organism of botulism is usually strongly proteolytic and its activity is hindered by the presence of high acidity. The toxin formed in milk, pasteurized or raw, is slowly destroyed by the milk types. When given by mouth instead of being injected, much more toxin is required to cause death.

In Edmondson, Thom, and Giltner's experiments we believe the small amount of toxin produced, the destruction of this toxin by the milk bacteria, and the larger quantity of toxin required to kill when given through the digestive tract explain the survival of the animals to which they fed "commercial" milk. The same line of reasoning will explain why the guinea pigs which we inoculated subcutaneously with raw milk, which had previously been inoculated with detoxified spores of *Clostridium botulinum*, died.

In the case of the deaths from botulism poisoning reported by Nevin (7) it is likely that the cottage cheese was heavily contaminated with the toxin producing organisms, and the persons dying probably ate large quantities of the cheese. Edmondson, Thom, and Giltner (4) were unable to produce poisonous cottage cheese by inoculating fresh, raw milk with *Streptococcus lactis* and "a few" detoxified botulinum spores, incubating the milk for two days at 20°C., and the cheese for four days at 16°C. after manufacture. The toxicity of this cheese was tested by feeding it to guinea pigs. These results are as would be expected in the light of our data.

It is believed that knowledge of the destructive action of intestinal and milk types of bacteria upon botulinum toxin will be of value to the canning and dairy industries of the country and the consuming public. The intestinal types with which we have worked are always present in market milk. We believe these results explain, in part, why milk and dairy products, which undoubtedly at times contain *Clostridium botulinum*, fail to cause poisoning.

SUMMARY

It has been shown that certain bacteria commonly found in milk have the power of destroying toxin produced by *Clostridium botulinum*. The data reported in this and a previous paper have shown that this destruction may be caused by *Streptococcus lactis*, *Lactobacillus casei*, *Bacterium coli*, *Bacterium communior*, *Bacterium aerogenes*, and *Proteus vulgaris*.

It is believed that these results explain in part the fact that milk and dairy products are seldom if ever agents in the dissemination of botulism.

The fact that *Streptococcus lactis* and *Lactobacillus casei* have the power to destroy botulinum toxin is of particular interest in connection with cheese, since in most types of cheese *Streptococcus lactis* is the predominating organism during the early stages of ripening, while *Lactobacillus casei* is one of the predominating organisms in all types of cheese during the later stages of ripening.

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BOVINE INFECTIOUS ABORTION

INCREASED PRODUCTIVITY OF AN ABORTION-FREE DAIRY HERD*

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INTRODUCTION

The clean herd under discussion here is the regular Connecticut Agricultural College herd, and comprises at this writing 115 head, of which 50 are milch cows, and the remainder young stock and bulls. This herd is made up of four breeds, Jersey, Guernsey, Ayrshire and Holstein, all at present pure-bred.

The earlier investigations[1] (1) conducted over a period of several years convinced the writers that effective barriers against the spread of the Bang abortion disease cannot be erected by ordinary methods of sanitation and temporary isolation. Continued experience with the agglutination and complement fixation tests and confidence in their results led them to the conclusion, however, that eradication is thoroughly feasible. Owing largely to the loss of the College dairy barn in 1919 which was not adequately replaced until 1924, the complete elimination scheme was not put into execution in the College herd until the winter of 1924–1925.

Almost from the beginning of the series of investigations on infectious abortion it became apparent to the writers that calves are not permanently infected at birth, or later during the milk feeding period, even though they are born of positive dams; and, therefore, that calves constitute no serious problem until they approach sexual maturity.

In anticipation of the completion of the new maternity and young stock barns the practice was begun in 1923 of removing

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[1] This is the thirteenth paper of a series on the subject of bovine infectious abortion. See bibliography (1).

female calves at weaning time (about six months) to quarters apart from the regular herd. Even though the heifers were later bred to non-reacting herd bulls which were used on both reacting and non-reacting cows, no difficulty was experienced in keeping the heifers abortion-free.

During all of 1924 a rather severe culling of the milking herd was under way. This culling involved the removal of animals which calved prematurely, and abortion reacting cows of mediocre milking capacity. As a consequence of this treatment of the herd, and because only an occasional animal was added, very few new reactors appeared in the herd during the nine months preceding the adoption of the full eradication scheme.

The new barn, which was separated from the main milking barn by a connecting storage building, was completed and put to practical use in November, 1924. The heifers that had been reared in separate quarters, of which several were well advanced in gestation, were at this time placed in the new barn. Although the policy of removing all reacting animals from the milking herd was not put into effect until February and March of the following year, 1925, infection did not spread to the new (young stock) barn. While the new barn contained maternity stalls, only non-reacting cows were placed in it.

Two of the heifers which had been reared in separate quarters became reactors in 1925. This was accounted for by the transfer to the mature herd of a few heifers after calving, before the reactors had been removed from the milking barn. These two newly reacting animals were, of course, removed with the other reactors at the time complete segregation was begun.

Some of the reactors were removed in February and the balance in March 1925, fifteen in all. They were taken to an old barn about three quarters of a mile distant from the main barns. This reacting herd was retained to maintain the general milk supply which was admittedly somewhat below normal because of the rather aggressive policy of disposing of reactors in the preceding nine months. Furthermore, some of the best producing cows were among the segregated reactors and they were needed as breeders to supply high grade calves for building up the clean herd.

Following the removal of all reactors, only one cow has been lost from the supposedly clean herd by becoming a reactor. This animal gave a suspicious reaction in May and June, 1925, and in the July test proved to be a complete reactor in the sixth month of gestation. This heifer had suffered from an acute attack of scours when only a few days old which resulted in a slight physical deformity. She had come from a positive dam and had reacted continuously until she was at least a year old. This heifer was one of the rare exceptions to the observed rule (Bul. No. 93) that heifers, no matter what their origin, are negative to the blood tests after they are from five to six months old. She was removed as a reactor, and later (September) aborted.

There can be no doubt that the rapid elimination of, and the apparently complete freedom from infection of this herd over a period of more than three years are due to stabilization or delimitation of the infection just before and at the time the reactors were removed.

Monthly tests were conducted on the clean herd for more than a year after complete segregation and following this the herd has been subjected to quarterly tests. The uniformity with which the results were negative, except for an occasional non-specific doubtful reaction, is further evidence of the value of the serological methods. These results are especially significant when we consider the high production and calving record of the herd.

In less than a year after the removal of all reacting animals the gross milk yield of the abortion-free herd was back to normal, due to the rapid addition of young, freshening cows. Since then the production record has been consistently greater than at any other time in the history of the herd.

The reacting herd has been continued, with some sales and new additions, for further study. The bulls of the non-reacting herd have been used with the reacting cows, but never without thorough disinfection of the sheath. The calves from the reacting herd have been transferred when one or two days old to the young stock barn of the clean herd. As calves from positive dams may carry the Bang bacillus temporarily from uterine exposure and by having ingested infected milk, they were treated ex-

ternally with disinfectant solution and placed in a small isolation pen for 50 to 60 days before they were allowed contact with other animals. More recently we have endeavored to bring new calves of the positive herd to the clean calf barn before they nursed. Such calves, as is now well known, react and continue to react negative, though they are from positive dams, and long continued isolation is not necessary. No feeding troubles have been encountered as the result of this practice.

ECONOMIC LOSSES FROM THE BANG ABORTION DISEASE

In an earlier Station Bulletin (Bul. No. 135) it was shown that the abortion reacting cows in the College herd gave a return of $44.01 less per cow per year over feed cost than the non-reacting animals. These figures were compiled from data covering an eleven year period in which reacting and non-reacting animals were in constant association with each other, and were based upon calf losses, milk yield, and depreciation in the value of animals from actual sales.

It should be understood that when the losses from premature calving (the calf itself) and from subnormal subsequent lactation were included the actual loss per cow for that period was much greater than $44.01. This figure is based upon the average yearly performance for full time in milk, which was practically four years per cow. Since the life time abortion rate among reactors was found to be 26.2 per cent (Bul. No. 123), the average number of abortions per cow was only about one during the four years residence as a milch cow. All gestations of less than 265 days were considered abortions.

Thompson (2) found that his segregated reacting Guernsey herd produced milk at a cost of 16.8 cents per quart, against 10.4 cents per quart for the non-reacting herd.

Sims and Miller (3) reported the milk production for two years by a herd that had been tested and the reactors and non-reactors segregated. The negative herd averaged 7343 pounds of milk per cow in 1923, and 6291 in 1924, and the positive 4544 pounds in 1923 and 3262 pounds in 1924. In the presumably clean herd one abortion occurred in 78 freshenings, and in the infected herd

10 cows out of 42 aborted. No statement of costs is given, except that the reacting herd was abandoned at the end of the second year because of the heavy losses.

Quite recently Newsom and Cross (4) reported abortion segregation results on the dairy and beef herds of the Colorado Agricultural College. They stated that it took ten and one-half months to eliminate the disease from the beef herd in which 16 animals or 43 per cent of the original herd were removed as reactors. A loss of $92.38 for each of the 16 reactors was estimated as the depreciation sacrifice. With the dairy herd eighteen and one-half months were required for complete eradication, with a sacrifice of 15 cows or 34 per cent of the original herd. The depreciation in this instance was estimated to be $70.55 per reacting animal. The reactors in the two herds were slaughtered, which accounts for the high depreciation. The premature calvings previously had ranged from zero to 17 per cent in the beef herd, and from zero to 23 per cent in the dairy herd, during the years 1917 to 1926, inclusive. In both of these herds slow conception constituted a serious menace at times.

Barnes (5) states that where 10 per cent of the herd aborts the equivalent of the entire herd is lost every five years. In the Storrs herd, which had an average of 19.5 per cent abortions over a period of 21 years, the equivalent of the whole herd was lost every four years. Barnes states further that the loss from abortion in Pennsylvania amounts to $5,000,000 annually.

RESULTS OF THREE YEARS' OBSERVATIONS ON THE ABORTION-FREE CONNECTICUT COLLEGE DAIRY HERD

It is the chief purpose of this paper to demonstrate the value of eradication of the Bang abortion disease, through increased financial returns.

Data will be presented under the following headings: (1) calving record; (2) live stock sales; (3) milk yield; and (4) other improvements.

Calving records

Calving data have been published from time to time in previous bulletins. These are summarized in table 1, together with the

more recent and unpublished figures for 1925, 1926 and 1927. Several distinctive and significant facts may be deduced from this table.

TABLE 1

Summary of calving conditions during the years 1904 to 1927, inclusive

YEAR	NUMBER OF COWS	BREEDING COWS WHICH CALVED		CALVING COWS WHICH ABORTED		REACTING COWS	
		Number	Per cent	Number	Per cent	Number	Per cent
1904					46.1		
1905					25.9		
1906					19.3		
1907					24.0		
1908					11.5		
1909					15.1		
1910					17.6		
1911					27.9		
1912					20.0		
1913					20.0		
1914	37	28	75.7	7	25.0	21	56.8
1915	39	31	79.5	2	6.5	16	41.0
1916	44	35	88.4	4	10.5	14	31.8
1917	45	34	77.7	3	8.6	12	26.7
1918	39	31	89.7	2	5.7	8	20.5
1919	46	37	82.6	12	31.6	16	34.8
1920	44	32	72.7	12	36.4	20	45.5
1921	45	40	88.8	10	20.0	21	46.7
1922	43	38	88.3	6	15.8	17	39.5
1923	46	39	80.5	11	28.2	21	45.7
1924	49	39	80.0	6	15.4	24	49.3
Average 21 years.....	43.4	35.0	80.5	6.8	19.5	17.3	39.8
1925	39	35	87.4	1	2.9	1	2.5
1926	49	49	100.0	3	6.1	0	0.0
1927	49	45	91.8	0	0.0	0	0.0

1. From 1904, when the first complete records were available and at which time presumably the infection was introduced into the herd, the abortion rate rose and fell at practically eight year intervals between the high peaks, running as high as 46.1 per

cent, and never lower than 5.7 per cent, in any calendar year up to the time of eradication.

2. The average number of premature calvings (before the 265th gestation day) during a period of twenty-one years through 1924 was 19.5 per cent.

3. After the reacting cows were segregated in 1925 one new reactor appeared in the supposedly clean herd (as previously described); since then no animals in the clean herd, nor any of their descendants in the herd, have reacted.

4. Up to the end of 1927 there have been 129 calvings in the abortion-free herd. Four of these were premature: this gives a rate of 3.1 per cent, which is not far from that observed previously in over 500 calvings of non-reacting cows (Bul. No. 123). Two of these abortions were satisfactorily accounted for, but there is no explanation for the one that occurred with a first calf heifer and another that occurred with a second calf young cow. Three of these aborting cows are breeding regularly now; the fourth is dead.

5. The breeding efficiency of the herd, that is the percentage of milking cows that calve in each calendar year, rose to 100 per cent in 1926, and in 1927 it was 91.8 per cent. Both of these are high peaks in the history of the herd. In 1926 one cow did not calve during the calendar year, but another calved twice during the year. A cow is classed as a breeding cow in every calendar year that she is in the herd after her first calving.

6. For the first time in the herd's history not a single premature calving occurred in the herd in 1927.

Perhaps the reader will wonder how the *reacting* herd has fared during the last three years period. About half of the original group remains and a few reacting cows, purchased here and there, have been added. There have been 29 calvings, ten or 34.5 per cent of which have been premature.

Live stock sales records

Obviously when the abortion disease is rampant in a herd the surplus stock sales must suffer. Table 2 presents these facts for

the twelve-year period beginning in 1916 and admits of some striking deductions.

1. Following an abortion storm, which abated in 1915 (see table 1), the stock sales were still light in 1916, but rose rapidly with the four good breeding years 1915, 1916, 1917 and 1918, when the total sales reached 32 head, at a value of $2,868.40 in 1919. During this time we were encouraged to believe that the disease would be stamped out of the herd, but in the latter part of 1919 the test showed unerringly that the disease again was spreading and that an

TABLE 2

Live stock sales from the College dairy herd

YEAR	TOTAL ANIMALS SOLD	TOTAL VALUE OF SALES	NUMBER OF CALVES VEALED	NUMBER OF COWS SOLD		NUMBER BULLS AS BREEDERS
				For meat	As producers	
1916	11	$653.00	1	4	1	5
1917	19	1,182.14	2	5	6	6
1918	13	1,532.50	1	3	7	2
1919	32	2,868.40	10	7	6	9
1920	22	1,995.00	3	12	3	4
1921	22	821.00	3	12	3	4
1922	23	781.00	8	9	5	1
1923	24	1,329.00	6	4	11	3
1924	24	1,059.32	9	5	5	5
1925	30	2,173.50	8	9	8	5
1926	33	1,113.00	14	7	4	8
1927	38	3,652.65	5	3	11	19
Total.....	291	$19,160.51	70	80	70	71

abortion storm was inevitable. It came and many of the second and third calf cows that had thus far escaped, as well as a majority of the first gestation heifers, became reactors, of which a goodly proportion aborted.

2. The slump in sales from 1920 to 1922 is particularly notable; also a decided shift in the proportion of cows sold as producers to cows sold to the butcher.

3. In 1923, 1924 and 1925 the sales of cows as producers were considerably increased as compared with those which went for beef, due to the disposal of some grade animals that had been

purchased for experimental feeding purposes, and which were no longer needed, and to the elimination of ordinary reacting cows in the latter part of this period preparatory to the complete elimination of reactors.

4. The male calves sold for veal and as breeders rose in 1926 to 22 (the highest point reached up to that time) but the sale of producing cows was still low because it was highly desirable to retain all satisfactory cows. Seven poor producers, most of which had been held longer in the herd than they really should have been went to the butcher during this year.

5. In 1927 a new high record for values, namely, $3,652.65, was established from a new high total of 38 animals sold. The shift to bull calves sold as breeders is noteworthy; this was made possible because there were so many good, normal calving cows. Since there was also a large number of surplus cows which were sold as producers, the sales netted an unusually good return.

Milk yield

The year by year milk yield of the herd is given in table 3. An explanation appears desirable, however, before presenting and discussing this table.

The number of milk cows included in the second column of table 3 does not agree precisely with the record of breeding cows in table 1, largely because some grades were occasionally introduced into the herd temporarily for use in feeding experiments, and since neither they nor their descendants became a permanent part of the herd they were omitted in the consideration of milk yield. This did not apply, however, to a few grades that had been bred in the herd and whose yields are included.

When a cow had once freshened she was ever afterwards regarded as a milking cow so long as she remained in the herd, and no deduction was made for dry periods. The seeming low average number of months in milk as given in the third column is due to the continual shifting of the herd. When a cow was sold in the latter part of March, for instance, she was credited with three months in that particular year, regardless of her stage of lactation, and even if she was dry during a part of or all of that time.

Likewise, when a heifer freshened for the first time she was considered as a milk cow for just that fraction of the year that she was in milk; for example, if she calved during the first part of September she was recorded as being in milk four months in that year. If a heifer freshened for the first time before the tenth day of the month it was called a full month; if she freshened between the 10th and 20th of the month it was recorded as a half month; and if she freshened between the 20th and the last day of the month this last fraction of the month was not counted. Likewise, although in reverse order, a cow that was sold was similarly recorded.

Since the animals were of different ages, production records were converted to full age by using figures based upon the studies of Gowen (6), Ragsdale and Turner (7), and of Fohrman (8).

Also, certain animals were on Advanced Registry test and necessitated corrections for these records, but we were at a loss to find accurate conversion factors to apply to this herd. Eckles (9), in a study of Advanced Registry records made in 1922 in the herds of the University of Minnesota, the University of Missouri, the University of Nebraska and the Connecticut Agricultural College, found that the herd record was equivalent to about 58 per cent of the Advanced Registry record for the same cow, but, because of certain specifications as to his requirements, many of the cows with Advanced Registry records in the Connecticut herd were not included at that time; hence we do not believe that this figure applies to the whole herd. Woodward (10) reported a conversion factor of 66.67 per cent in the Government herd at Beltsville, where the cows were in box stalls while on test. Fohrman (11) claimed that an approximate increase of 11.5 per cent is obtained in a retest by virtue of the development of the cow. Riford (12), in a large commercial herd, found that increasing the number of milkings to three times a day resulted in an increase of about 15 per cent in the yield.

Only a very small number of the test cows in the Connecticut Agricultural College herd have been milked four times a day, and none throughout a complete registry record in recent years. Also, no cow has been held throughout the entire period in a box stall,

and the great majority have not been in a box stall at all except at calving time. Thus, it seems that in this herd the herd yield would be nearer 70 per cent of the Advanced Registry yield for all cows. This factor was used, therefore, in computing yields during the period when the cows were being milked more than twice daily. The number of cows maintained on Advanced Registry test has been fairly uniform.

TABLE 3

Production data presented by calendar years 1915–1927

YEAR	NUMBER OF COWS	AVERAGE MONTHS IN MILK	ACTUAL AVERAGE YIELD PER COW		ACTUAL TOTAL MILK YIELD	CORRECTED FOR AGE AND ADVANCED REGISTRY YIELD PER COW		NUMBER OF 12-MONTH COW YEARS	12-MONTH COW YIELD FROM CORRECTED YIELD		PER CENT PREMATURE CALVINGS
			Milk	Fat		Milk	Fat		Milk	Fat	
					pounds						
1915	37	8.98	6,981	293.4	258,305	6,816	293.4	27.7	9,104	391.8	6.5
1916	39	7.70	5,765	232.0	224,828	5,516	226.5	25.0	8,591	352.8	10.5
1917	39	8.86	7,015	275.8	273,592	6,939	274.6	28.8	9,397	371.9	8.6
1918	34	8.15	6,279	258.0	213,471	6,195	258.3	23.1	9,127	380.4	5.7
1919	45	7.20	4,678	199.7	210,539	5,214	221.2	27.1	8,664	367.6	31.6
1920	48	8.24	4,917	201.2	236,009	5,169	217.9	33.0	7,528	317.0	36.4
1921	46	7.66	5,543	212.4	254,955	5,598	215.7	29.4	8,768	378.7	20.0
1922	41	8.13	6,309	246.3	258,682	6,315	246.3	27.8	9,316	363.3	15.8
1923	36	8.93	6,528	256.2	235,021	6,044	242.3	26.8	8,158	325.6	28.2
1924	47	7.19	6,503	259.2	305,663	6,099	254.8	28.2	10,108	425.2	15.4
1925	36	7.50	6,788	277.3	244,375	6,458	261.2	22.5	10,314	417.3	2.9
1926	48	7.49	7,200	278.8	343,578	7,064	271.2	30.0	11,314	434.5	6.2
1927	43	8.84	8,045	328.7	345,950	7,152	300.1	31.7	9,712	407.6	0.0

Table 4 is derived from table 3. Special attention is called to these data. The average fat production given in the column next to the last in table 3 is repeated in the second column of table 4. But the milk yield cannot be so satisfactorily presented, because the four breeds of cows in the herd are not maintained in the same numerical relationship from one year to another. Therefore, Table 4, in which the milk yield is calculated from the fat yield, gives the values in terms of milk yield for a Holstein, an Ayrshire, a Guernsey, and a Jersey herd.

Corrections have been made for differences in breed and in age, and for Advanced Registry test. There are still other variable factors for which there are no known means of correction. For instance, an effort has most naturally been made to improve the herd. No correction can be made for this difference, whatever it may be, but it can be said that there has been no bull in use at any period that has proved to be outstanding in transmission of yield qualities, and with four breeds involved no single bull has been able to create a dominant influence upon the whole

TABLE 4

Calculated milk yields from corrected per cow year fat yield

YEAR	PER COW YEAR FAT YIELD FROM TABLE 3	EQUIVALENT MILK YIELD CALCULATED FROM FAT YIELD				PER CENT PREMATURE CALVINGS
		3.45 per cent milk	4 per cent milk	5 per cent milk	5.35 per cent milk	
	pounds	*pounds*	*pounds*	*pounds*	*pounds*	
1915	391.8	11,357	9,795	7,836	7,323	6.5
1916	352.8	10,226	8,820	7,056	6,594	10.5
1917	371.9	10,779	9,298	7,438	6,951	8.6
1918	380.4	11,026	9,510	7,608	7,110	5.7
1919	367.6	10,655	9,190	7,352	6,871	31.6
1920	317.0	9,188	7,925	6,340	5,925	36.4
1921	378.7	10,977	9,468	7,574	7,078	20.0
1922	363.3	10,530	9,083	7,266	6,791	15.8
1923	325.6	9,438	8,140	6,512	6,086	28.2
1924	425.2	12,325	10,630	8,504	7,948	15.4
1925	417.3	12,096	10,433	8,346	7,800	2.9
1926	434.5	12,594	10,863	8,690	8,121	6.2
1927	407.6	11,861	10,190	8,152	7,619	0.0

herd. Furthermore it is admitted that it has been our aim to improve the feeding and management in the herd. During the past seven years the grain rations have been very much the same but pasture improvement and somewhat more liberal hay feeding, when pastures were short, have without doubt had some influence. However, the year to year yield seems to show no precise evidence of an influence from either of these factors.

The following deductions are made from tables 3 and 4.

1. The yield in 1915 was 391.8 pounds of fat per cow, equivalent to 9795 pounds of 4 per cent milk. The yield fluctuated

between this amount and 317.0 pounds of fat. In 1923 the yield was 325.6 pounds. There is not the slightest indication of an upward trend through these years that may be attributed to an improvement in breeding and management.

2. In 1924, when the aborting animals were promptly removed and the herd thus placed on a practically abortion-free basis, the average production was 425.2 pounds of fat, equivalent to 10,630 pounds of 4 per cent milk. Thus, for the first time the herd

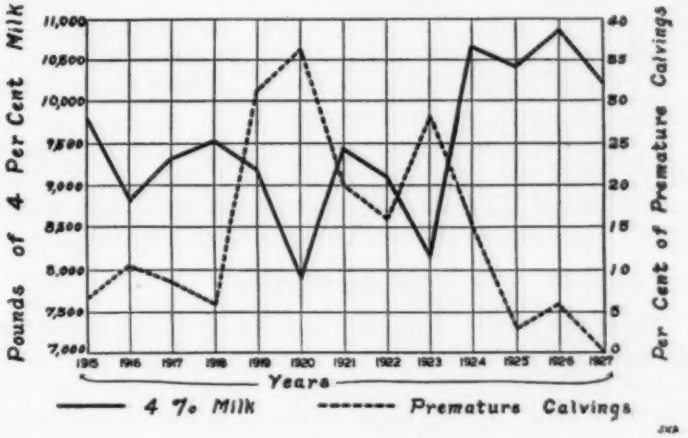

FIG. 1. ANNUAL PER COW MILK YIELD AND PREMATURE CALVING RECORD, 1915–1927

yield had gone beyond 400 pounds of fat and 10,000 pounds of 4 per cent milk.

3. From 1924 to 1927, inclusively, the yield has remained above 400 pounds of fat and 10,000 pounds of 4 per cent milk.

4. The equivalent milk yield of 3.45 per cent test reached 12,000 for the first time in 1924 and remained above this figure, except in 1927 when the yield was just below 12,000; the equivalent milk yield of 5 per cent test went above 8000 for the first time in 1924, and has remained above this figure; and the equivalent milk yield of 5.35 per cent test went above 8000 in 1926, and has been close to this level in each of the last four years.

5. For nine years before 1924 the average (direct average) was 361.0 pounds of fat. In the past four years the average has been 421.2 pounds of fat. For the nine years before 1924, the average yield per year of 4 per cent test milk was 9025, and in the past four years it has been 10,530 pounds.

6. Beginning in 1924, when all aborting animals were removed, and continuing through 1927, during the last three years of which the herd has been free from the Bang abortion disease, the herd has averaged 1505 pounds more per cow per year than during the previous nine years. This may at first thought appear to be a disappointing improvement, but it must be considered:

a. That during the nine years covered by these data all of the animals, of course, were not diseased.

b. Fifteen hundred pounds of milk, or 60.2 pounds of butterfat, represent the cream of the profit on a cow. At $3.50 per hundred weight (7½ cents per quart) this amount of milk is worth $52.67. In a herd of 20 cows it has a value of $1053.40.

Other improvements

One of the results of an abortion-free herd that has already appeared and which should be even more noticeable in another year or two is the improvement in the appearance and the soundness of the herd. It is no longer necessary to keep all cows simply because they will produce some milk. As a consequence, the herd is now more valuable than it has ever been and we believe it is destined to reach a still higher plane of production.

In addition, it is our belief that there has been some reduction in retained afterbirth and in the number of services per conception, but the differences may not be very pronounced, and more complete data are needed before these points are definitely determined. In an earlier publication (Bul. No. 135) it was pointed out that there was apparently not a great difference in favor of the non-reacting cows in these two respects.

SUMMARY

In looking forward, in 1923, to the establishing of an abortion-free herd at the Connecticut Agricultural College, the practice

was instituted of protecting maturing heifers by removing calves at six months of age, whether they were from abortion reacting or non-reacting dams, to separate premises a half mile distant from the main herd.

In order to stabilize the infection in the main herd the policy was adopted in 1924 of disposing of all aborting (premature calving) cows.

In February and March of 1925 the fifteen remaining reactors were removed from the herd and segregated. In July, 1925, one of the supposedly clean young cows reacted. She was promptly removed, and aborted two months later. Since that time not a single animal has reacted to the blood tests except young calves from the segregated reacting cows. This outstanding success in so completely removing the foci of infection in the initial separation was undoubtedly promoted by the process of stabilization or delimitation of the disease through the preliminary measures employed.

The abortion rate (premature calvings prior to the 265th day of gestation) which had varied in intensity during the years from 1904 to 1924, averaging 19.5 for each 100 calvings during this time, dropped to 2.9 per cent in 1925. It was 6.1 per cent in 1926, and 0.0 in 1927. The average premature calving rate during the past three years was 3.1 per cent, this figure being substantially the same as that previously reported by us from over 500 calvings of non-reacting cows.

The number of cows calving in each calendar year, which averaged 80.5 for each 100 cows from 1904 to 1924, was 87.4 in 1925, 100 in 1926, and 91.8 in 1927, the last three years being the period in which the herd has been free from abortion reactors.

The live stock sales reached a high peak in 1927, due not only to a larger number of surplus animals but also to the larger proportion of more valuable breeding and producing animals available from the non-reacting herd.

The milk yield of the non-reacting herd has averaged 1505 pounds more of milk testing 4 per cent per cow per twelve-month year than the previous herd which contained both reacting and non-reacting cows. During the last four years the average yield

of the herd has been 10,530 pounds of four per cent milk, while for nine years prior to that the average was 9025 pounds.

Finally, the appearance of the herd unquestionably shows an improvement as a result of the increased opportunity for culling that is afforded in an abortion-free herd.

In conclusion, the writers wish to express their appreciation to Dr. Charles A. Slanetz, Mr. George Brigham and Mr. Charles Oliver for their valuable assistance and coöperation.

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A DEFECT IN MILK DUE TO LIGHT*

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In cold weather the "outdoor icebox" is used extensively, especially by apartment dwellers. Milk, with other food, is placed in a more or less open box on the window sill or even out on the sill. This milk sometimes develops an off flavor that has been described as a "cardboard" taste, for which the consumer is more likely to blame the milk distributor than himself.

The appearance of a cardboard flavor in milk is common and may be due to any of several causes. The term "cardboard" is itself indefinite and is undoubtedly applied to different flavors and odors which result from changes in the butterfat. Some flavors are designated as "flat" and others are slightly metallic, although most of the flavors which result from oxidation of the fat may be termed "tallowy" or "cardboard."

Hunziker (2), (3), has reviewed work by himself and others on the effect of various metals on the flavor of milk and other dairy products. He calls attention to the fact that metals from various parts of farm and milk plant equipment may enter the milk and act as oxidizers or catalyzers with the production of metallic and other off flavors. Flat or cardboard flavors may result in the action on milk fat.

Hammer and Cordes (1) have reported that the action of direct sunlight on milk and cream produces a definitely tallowy flavor on sufficient exposure and a distinct off flavor with less exposure. Off flavors were observed in certain milk samples after an exposure of only ten minutes; and tallowiness appeared after exposure for as short a time as forty-five minutes. Exposure to air apparently aided the development of the defect. The use of brown bottles was shown to prevent the appearance of the bad flavors, but the brown bottles had other disadvantages which more than offset

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this one advantage. Season of the year had little influence on the development of the flavor. Sunlight had a greater influence on milk of low butterfat content than on milk of high butterfat content.

It is generally supposed, however, that while direct sunlight is very active in causing these changes in milk, that diffuse light acts very slowly and may take days to bring about undesirable changes in flavor. In the experiments here reported the milk was never exposed to direct sunlight, but only to diffuse light in a north window which was never reached by the sun.

The defective taste which is being here discussed is not metallic but resembles cardboard, and the odor is like that of a drying linseed oil accompanied by a sweetish odor. As an after-taste there is in the back of the mouth a puckering sensation which is probably due to free fatty acids.

In this investigation it was apparent that light probably played a part in the production of the defect. To demonstrate that light was essential, in all the work described below, duplicate samples were prepared, of which one was exposed to daylight and the other was placed in the dark in a well-aerated metal container. In all cases the milk samples kept in the dark showed no evidence of a cardboard odor or flavor, even after seven to nine days at near freezing temperatures, whereas the samples kept in light at the same temperature developed the characteristic cardboard odor and taste after twenty to forty-eight hours of which eight to twenty-six hours were daylight. Exposure to diffuse daylight apparently instigates an oxidation process which continues in the dark. The samples were kept in flasks or bottles which were stoppered with cotton or covered with paper or a sterile glass beaker, so that no cardboard taste could come from a cardboard cap. The samples were never exposed to direct sunlight but were placed in a north window in diffuse light. Incubation temperatures were, for the most part, just above freezing. No difference in results was noted whether soft-glass milk bottles were used or hard-glass (Pyrex) flasks.

That the defect develops in the cream and not in the skim milk was shown by exposing skim milk, whole milk, and cream

from the same milk sample. The skim milk developed no cardboard taste, whereas the whole milk and cream showed the defect.

To determine whether the cardboard taste and linseed-oil odor might result from the absorption of part of the taste and odor of ordinary tallowy butterfat, the fat from one of the milk samples was separated and exposed to direct sunlight for a few hours. It was then held at 50°C. until a marked tallowiness developed when fresh skim milk was added and the mixture thoroughly shaken. The same odor and taste that was in the tallowy butterfat persisted in the reconstituted milk and no resemblance to the cardboard taste or linseed-oil odor was noted.

The effect of heating the milk on the development of the defect was shown by a comparison of raw samples and those pasteurized at 62.5°C. for one-half hour. The pasteurized samples usually developed the cardboard taste more rapidly than the raw samples. When the milk was heated in an Arnold steamer or was sterilized in the autoclave, the defect appeared a little more slowly. In the case of milk sterilized in the autoclave the action of both enzymes and bacteria was eliminated. These factors, however, may play a small part in the development of the defect in raw or pasteurized milk.

The resemblance of the odor of the milk with a cardboard taste to that of drying linseed oil indicated that the odor might be due to the oxidation of one of the more unsaturated fatty acids. Oleic acid, the chief unsaturated fatty acid of milk fat, is not so readily oxidizable as the more unsaturated linoleic and linolenic acids. It has been reported by Hunziker, Mills and Spitzer (4) that the characteristic chemical constants of milk fat vary when corn oil, linseed oil, and cottonseed oil are fed to cows, and that the iodine number, in particular, is increased by these feeds. Therefore it was thought that these oil feeds might so influence the milk fat that it would be more susceptible to oxidation. An experiment was conducted with the milk of cows selected from the experiment farm of the Bureau of Dairy Industry at Beltsville, Maryland. These cows were being fed as follows: Cows 1 and 2 received no oil feed; cows 3 and 4 received a heavy ration of cottonseed meal as their only oil ration; and cows 5 and 6 received

a heavy ration of linseed cake. The defect was found to be just as pronounced in the milk from cows 1 and 2, which received no oil feed, as in the milk from cows 3, 4, 5, and 6. The milk samples were obtained from the milk pails immediately after milking and did not pass over a cooler or through any other metal equipment.

DISCUSSION

The defect which has been described is apparently of very general occurrence for it developed in all milk samples from a number of sources. The remedy is to keep the milk in the dark even when the temperature is near freezing. Consumers should be so advised.

It is apparent that exposure to diffuse light for a few hours so shortens the induction period of the milk fat that oxidation may begin with consequent production of undesirable odors and flavors. The few hours of exposure to light apparently starts a process which continues in the dark and is accelerated by more daylight. Increased quantities of unsaturated fatty acids other than oleic acid seem unnecessary for the action. The odor and taste after the first twenty to forty-eight hours is that of an oxidized fat and gives no evidence of rancidity, although after exposure for a number of days rancidity may also begin to appear. Although the heavy glass of the milk bottles screens out the ultraviolet rays, it allows the passage of the active longer rays which exert a catalytic action. The milk fat apparently is made more easily oxidizable by the heat of pasteurization. The presence of a metal catalyst from equipment or other source would undoubtedly assist the catalytic action of daylight.

SUMMARY

A "cardboard" taste and "linseed-oil" odor develop in whole milk which has been exposed to diffuse daylight for eight or more hours at about freezing temperature. The light apparently acts as a catalyst in the oxidation of the milk fat. The defect develops more rapidly in pasteurized than in raw milk. The presence of neither enzymes nor bacteria is necessary for the

reaction. The defect develops no more rapidly in milk from cows fed heavy rations of cottonseed meal or linseed cake than in milk from cows receiving no oil feed.

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SOME OBSERVATIONS ON THE CONSISTENCY OF CREAM AND ICE CREAM MIXTURES*

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INTRODUCTION

In a previous paper Bateman and Sharp (3) gave the results of an investigation of the apparent viscosity of milk and some of the important factors which influence it. The object of this series of experiments was to study the "viscosity" of cream and ice cream mixtures in order to gain some idea of the reliability of the results obtained with viscometers which operate on the liquid with a single shearing force. A search of the literature showed that no work had been reported on the plasticity of cream or ice cream mixtures with the exception of a short paper by Masurovsky (9) who concluded that ice cream mixtures were plastic but that 20 and 40 per cent fat creams were not.

Bingham (4) has greatly advanced our knowledge of the laws of flow by showing the main difference between viscous and plastic flow through capillary tubes. The slightest pressure or force will cause the flow of viscous liquids although in some cases the flow will be extremely slow. The flow of viscous liquids is directly proportional to the force producing the flow. Plastic substances, on the other hand, have some of the properties of solids, that is, they are able to hold their shape under small shearing forces but are readily made to flow under higher shearing forces. The shearing force which is theoretically just necessary to start the flow of a plastic substance is called the "yield value." If at the higher forces producing the flow of a plastic substance, the yield value is subtracted from the total force then the flow of the plastic substance is directly proportional to this corrected force.

This relationship is brought out by equation (1) which is the

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equation for the determination of the consistency of a plastic substance by means of its flow through a capillary tube. ξ the consistency of a plastic substance corresponds to η the viscosity of a liquid.

$$\xi = \frac{\pi r^4 g (P - p) t}{8 l v} \tag{1}$$

Where r is the radius of the capillary, l its length, v the volume flowing through the capillary in the time t, P the total pressure in grams per square centimeter producing the flow, p the yield value pressure and g the gravitational constant. The only difference between the fundamental equation for viscous flow and that for plastic flow is the substitution of $(P - p)$ in the later for P in the former. It can readily be seen from equation (1) that if p equals zero then the substance is viscous. On the other hand if p has a value greater than zero the substance under investigation is plastic.

In studying the physical properties of milk, cream, or other colloidal suspensions, it is desirable to determine by means of observations made at several sufficiently high shearing forces, whether or not the coefficient of resistance to flow is independent of the force producing the flow. If the coefficient is found to be constant the material may be classed as truly viscous, if not, the material is only apparently viscous or may be truly plastic.

Bateman and Sharp (3) showed that even the viscosity coefficient of milk was dependent on the shearing force. This effect can be best brought out by reference to figure 2 of their paper in which the viscosity is plotted against the pressure producing the flow. It is seen that a truly viscous liquid such as the 20 per cent sucrose solution gives a viscosity-pressure curve that is a straight line parallel to the pressure axis; in other words the viscosity coefficient is independent of the pressure. This is not the case with milk since its viscosity coefficient shows a decrease as the force producing the flow is increased. This means that the so-called viscosity of milk cannot be expressed accurately by a single value.

A viscous liquid may be distinguished from a plastic substance by plotting the rate of flow through a capillary tube in cubic

centimeters per second at varying pressure. If the kinetic energy correction is negligible, the graph representing a viscous liquid will be a straight line passing through the origin. On the other hand if the straight portion of the line extrapolated intersects the pressure axis to the right of the origin as shown in figure 2 of this paper, the substance is plastic.

In this paper the rate of flow of cream and ice cream mixtures through a capillary tube was studied. It will be noted in figure 2 that at higher pressures, a straight line expresses the relation between the rate of flow and the pressure, and that this straight line extrapolated intersects the pressure axis to the right of the origin. The slope of the line times the constant of the plastometer gives the consistency, and the point of intersection on the pressure axis gives the yield value. At lower pressures, the pressure-rate of flow line curves towards the origin. This discrepancy is probably due to the partial breaking down of a structure in the dispersed phase and is overcome by using higher pressures, since under these conditions the relation between the rate of flow and shearing force becomes linear.

EXPERIMENTAL

Apparatus

The Bingham (4) plastometer has been used by various workers and the one used on this work was a modification of it. Figure 1 shows a diagram of the plastometer. It is similar to the one used by Sharp (11), except that the instrument was modified so as to better adapt it to the conditions of the problem.

The reservoir *A* consisted of a piece of glass tubing with an internal diameter of 2.5 cm., and a length of about 12 cm. It was closed at each end by means of one hole stoppers. A short piece of glass tubing *F*, was inserted into the top stopper and this served as a connection between the plastometer and the pressure line. Into the lower stopper, a capillary tube was introduced which projected through the rubber stopper *D*, into the receiving vessel *B*. The reservoir stoppers were firmly held in place by means of a clamp which consisted of two metal plates placed at

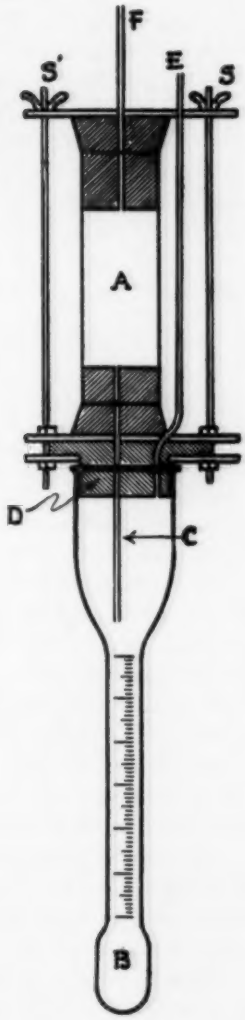

FIG. 1. DETAILS OF THE PLASTOMETER

the top and bottom of the tube *A*. These plates were kept in place by two bolts with winged nuts at the top. At the bottom was a metallic plate with a large hole in the center, through which the special rubber stopper *D* projected. The bolts pierced this plate, and firmly held the stopper *D* in position. The glass tube *E* served as an outlet for air which was displaced, when the liquid was forced from the reservoir *A*, through the capillary *C* into the receiving vessel *B*. This plastometer was substituted for the viscometer in the apparatus described by Bateman and Sharp (3).

General procedure

A definite amount of cream, which had been previously brought to the working temperature (25°C.) and thoroughly mixed, was poured into *A*. The stopper was then replaced and clamped in position. A receiving vessel *B* of desired volume was inserted over the special stopper *D*. The opening *E* was closed by means of a piece of rubber tubing and a clamp. This was done to prevent the leakage of cream into vessel *B*, before and after the determination. The completed apparatus was quickly inserted into the water bath and connected to the pressure line. The pressure, as indicated by the manometer, was adjusted prior to making the determination. The clamp was removed from *E*, and the pressure was turned on by means of the three-way stopcock. The cream was allowed to flow until it filled the receiving vessel *B* somewhere along the constricted, graduated portion. When the cream was thin, or when higher pressures were used, a receiver having a larger volume was attached to the apparatus. This made it possible to allow more cream to flow through the capillary, and thereby, kept the length of the time of flow within desirable limits. At the end of the run, the stopcock was turned so as to connect the plastometer with the atmosphere of the laboratory. When the pressure was released from the instrument, the watch was stopped and the time of flow *t*, recorded. The volume of flow *v*, was noted directly from the graduations on the receiver.

Calibration

It was first necessary to select a number of capillary tubes of uniform bore, cut these into appropriate lengths, and determine by trial runs which one was best suited to the properties of the samples of cream to be studied. The radii of the capillaries were measured by the microscopical method. These measurements were checked by filling the bore of the capillary with mercury, and weighing. From the weight and length of the mercury thread, the radius of the capillary was calculated. Bingham (4) fully described this method.

The radius of the capillary used in this work was found to be 0.02678 cm. with the microscope, and 0.02675 cm. with the mercury thread, which gives an average of 0.02676 cm. This capillary was next ground down until its length was equal to 10 cm.

In the introductory part, it was noted that the rate of flow of plastic substances might be approximately expressed by equation (1).

It is usually customary to express the experimental data in terms which are supposedly independent of the dimensions of the capillary. The pressure, P, and the yield value, p, were changed to dynes per square centimeter, and are represented by F and f, respectively. The value of F, or the shearing force on the walls of the capillary is given by the following equation:

$$F = \frac{rPg}{2l} \tag{2}$$

Substituting the values from (2) into equation (1) we obtain:

$$\xi = \frac{\pi r^3 (F - f)}{4V} \tag{3}$$

Where V is the flow in cubic centimeters per second, but, for the same capillary

$$\frac{\pi r^3}{4} = C \text{ (constant)} \tag{4}$$

which was numerically equal to 1.505×10^{-6} for the capillary used here.

Substituting this value of C into equation (3) gives the working equation for these experiments.

$$\xi = \frac{1.505 \times 10^{-5} (F - f)}{V} \tag{5}$$

In plasticity measurements the kinetic energy corrections are usually omitted, since they are ordinarily negligible under the experimental conditions used for studying plasticity.

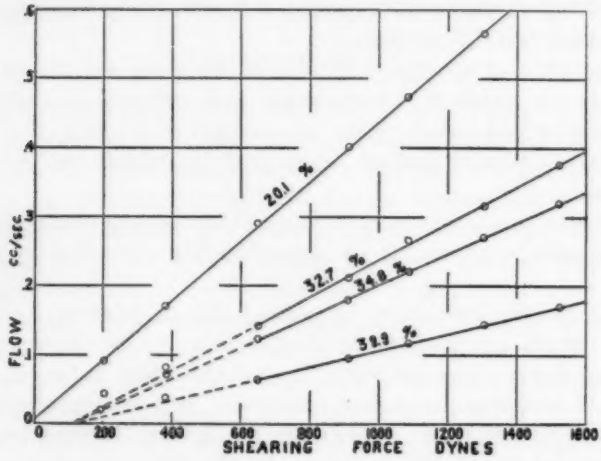

FIG. 2. THE RATE OF FLOW OF PASTEURIZED CREAM OF VARYING FAT CONTENT UNDER VARYING SHEARING FORCE

The total pressure, P, which was being used in any given determination of plasticity, was found by adding the pressure due to the average hydrostatic head of the cream in the plastometer to that indicated by the manometer. The pressure in each case was then changed to the shearing force in dynes by multiplying by the factor 1.312 obtained by substituting the values for r, g, and l in equation (2).

The yield value, f, was determined by the method of least squares, and checked graphically.

Effect of fat content and aging on the plasticity of pastuerized cream

The results obtained are given in table 1. The effect of increasing fat content on the plasticity constants is expressed graphically in figure 2. It is seen that as the fat content increases so also does the yield value and the consistency. The sample containing 20.1 per cent fat in the figure apparently gives a small negative yield value. This was due to the fact that the rate of flow was so great that the kinetic energy correction which was assumed to be negligible actually exerts an appreciable effect. If a correction is made for kinetic energy a small positive yield value is obtained. This was the only sample which did not show a definite positive yield value in the graph. The data for the sample containing 34.8 per cent fat was taken from another experiment in which case the cream was pasteurized twice. As the fat concentration increases the yield values are 8, 110, 109, and 120 while the consistency values are 3.21, 5.64, 6.66, and 12.4 $\times$ 10^{-2}. The last three-yield values are very nearly the same while the consistency of the same samples increases considerably. Since these were samples of pasteurized cream, the fat globules were probably not clumped to any considerable extent and more reproducible results could be obtained than with raw cream.

Various investigators have shown that pasteurized cream increases only slightly in viscosity on aging. The effect of aging on two samples is shown in table 1. Aging caused a slight increase in both the yield value and in the consistency.

Effect of heating on the plasticity of cream

Woll (16) and Babcock and Russell (1) (2) have shown that heating cream decreases the viscosity and breaks up the clusters of fat globules. Babcock and Russell attributed the decrease in viscosity to the breaking up of the clumps of fat globules. In our experiment 44.9 per cent fat cream was heated at 73°C. for thirty minutes. This temperature was chosen because such heating destroys the clumping of the fat globules while at the same time leaves the plasma with the same viscosity as unheated

TABLE 1

Effect of fat content and aging on the consistency of pasteurized cream determined at 25°C.

SAMPLE NUMBER	DAYS OF AGING	FAT CONTENT	SHEARING FORCE DYNES (F)	YIELD VALUE (f)	$(F-f)$	FLOW V	CONSISTENCY $\frac{C(F-f)}{V}$
		per cent				*cc. per second*	
1†	0	20.1	1,305	8	1,297	0.566	3.20×10^{-3}
			1,087		1,079	0.473	3.22
			912		904	0.401	3.22
			649		641	0.290	3.20
			383		375	0.171	3.22
			205		197	0.092	3.19
Average							3.21
2	0	32.7	1,522	110	1,412	0.376	5.65×10^{-3}
			1,306		1,196	0.316	5.70
			1,087		977	0.266	5.52
			911		801	0.213	5.66
			651		541	0.143	5.69
			383*		273	0.083	4.96
			206*		96	0.044	3.28
Average							5.64
2	4	32.7	1,522	124	1,398	0.318	6.62×10^{-3}
			1,305		1,181	0.263	6.76
			1,087		963	0.216	6.71
			911		787	0.177	6.69
			640		525	0.120	6.59
			383*		259	0.071	5.48
			204*		80	0.037	3.27
Average							6.67
3	0	39.9	1,524	120	1,404	0.171	12.4×10^{-3}
			1,307		1,187	0.145	12.3
			1,089		969	0.117	12.5
			912		792	0.096	12.4
			649		529	0.065	12.3
			383*		263	0.038	10.4
			196*		76	0.021	5.7
Average							12.4
3	3	39.9	1,503	184	1,319	0.153	13.0×10^{-3}
			1,305		1,121	0.130	13.0
			1,087		903	0.103	13.2
			912		728	0.083	13.2
			649*		465	0.055	12.7
			384*		200	0.033	9.1
Average							13.1

* Not used in the average or in determining the yield value.

† Corrected for kinetic energy.

plasma as was shown by Whitaker, Sherman and Sharp (14). The yield value before heating was 262 dynes and the consistency was 37.9 $\times$ 10^{-2}, after heating the yield value was 226 dynes and the consistency was 26.4 $\times$ 10^{-2}. Heating under these conditions decreased both the yield value and the consistency, the latter decreasing the most. This decrease in plasticity must be attributed to an effect of the fat; mainly a less clumped condition of the fat globules.

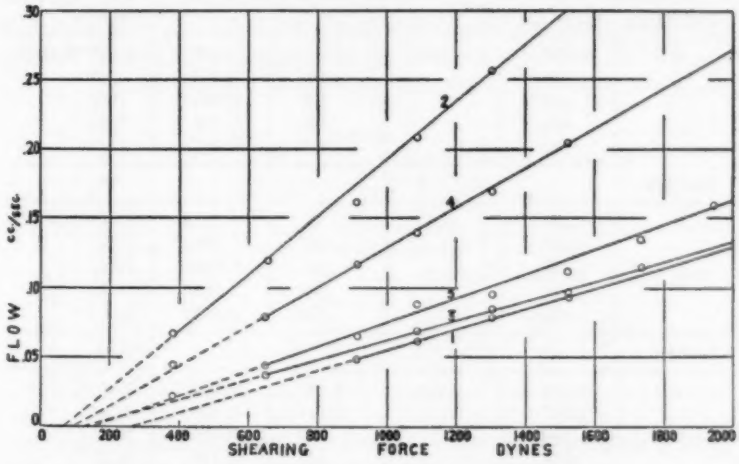

FIG. 3. THE RATE OF FLOW OF DIFFERENT ICE CREAM MIXTURES UNDER VARYING SHEARING FORCE

There is little doubt that the effect of heating would have been more pronounced if the plasticity constants had been determined at a lower temperature.

Effect of homogenization on the plasticity of pasteurized cream

Buglia (5) and Bateman and Sharp (3) have shown that homogenization increases the viscosity of whole milk while it produces no appreciable effect on the viscosity of skim milk. Wiegner (15) also showed that the viscosity of whole milk was

TABLE 2

Consistency of several ice cream mixtures at 25°C.

ICE CREAM MIXTURE NUMBER	SHEARING FORCE DYNES (F)	YIELD VALUE (f)	($F-f$)	FLOW V	CONSISTENCY $\frac{C(F-f)}{V}$
				cc. per second	
1	1,524	264	1,260	0.0931	20.4×10^{-2}
	1,308		1,044	0.0772	20.4
	1,089		825	0.0608	20.4
	914		650	0.0481	20.3
Average					20.4
2	1,307	66	1,241	0.257	7.27×10^{-2}
	1,089		1,023	0.208	7.40
	913*		847	0.162	7.87
	654		588	0.119	7.44
	383		317	0.067	7.12
Average					7.31
3	1,737	126	1,611	0.1154	21.0×10^{-2}
	1,523		1,397	0.0972	21.6
	1,307		1,181	0.0837	21.2
	1,088		962	0.0680	21.3
	649		523	0.0375	21.0
Average					21.2
4	1,524	93	1,431	0.204	10.6×10^{-2}
	1,307		1,214	0.169	10.8
	1,088		995	0.139	10.8
	913		820	0.117	10.6
	649		556	0.079	10.6
	383*		290	0.0448	9.7
Average					10.7
5	1,948	150	1,798	0.151	18.9×10^{-2}
	1,735		1,585	0.135	17.7
	1,522		1,372	0.112	18.4
	1,305		1,155	0.095	18.3
	1,088		938	0.078	18.1
	912		762	0.064	17.9
	649		499	0.0434	17.3
	383*		233	0.0225	15.6
Average					18.1

* Not included in the average or in determining the yield value.

increased by homogenization. Bateman and Sharp (3) found that the fat globules in homogenized milk were not clumped. Mortensen (10) and Sherwood and Smallfield (13) stated that the fat globules in homogenized cream were clumped and the latter concluded that the extent of the clumping was related to the viscosity.

The effect of homogenization on the plasticity constants of a sample of 34.8 per cent fat cream was determined. The yield value before homogenization was 109 dynes and the consistency was 6.66×10^{-2}, and after homogenization the yield value increased to 336 dynes while the consistency increase to 52.2×10^{-2}. Homogenization increased the yield 3.1 times while it

TABLE 3

Approximate composition of the ice cream mixtures of table 2

MIXTURE NUMBER	FAT	MILK-SOLIDS —NOT-FAT	SUGAR	GELATINE	YIELD VALUE	CONSISTENCY
	per cent	*per cent*	*per cent*	*per cent*		
1	11	11	14	0.3	264	20.4×10^{-2}
2	11	6	14	0.3	66	7.31
3	11	11	18	0.3	126	21.2
4	11	11	10	0.3	93	10.7
5	12	11	14.5	0.4	150	18.1

increased the consistency 7.8 times. Thus homogenization produces a tremendous increase in the resistance of flow.

The plasticity of ice cream mixtures

The plasticity constants for 5 different ice cream mixtures are presented in table 2 and figure 3. The composition of the mixtures is given in table 3. The compositions given in table 3 are only approximate and the previous treatment of the mixtures is not known so that no attempt should be made to correlate the plasticity constants with the composition of the ice cream mixtures. The data are presented to show the relative magnitude of plasticity constants which might be expected for ice cream mixtures. The plasticity data given for these ice cream mixtures corresponds to what Leighton and Williams (7) would probably call "apparent viscosity" at 25°C.

DISCUSSION

The plasticity experiments reported in this investigation were all carried out at 25°C. and not at near the freezing or storage temperature, because the temperature factors of the measurement could be controlled more accurately at 25°C.

The graph in figure 2 indicates that 20.1 per cent fat cream is viscous since the yield value was apparently zero. This is not actually true but appears so because the rate of flow was so great that the kinetic energy error was appreciable. After making this correction 20 per cent cream shows a small yield value and is therefore plastic. The capillary used was chosen because it could be used with the more plastic samples and therefore it was not suited for the measurement of the flow constants of the 20 per cent pasteurized cream. The possible effect of the diameter of the capillary on the plasticity constants was not determined.

The expected influences of aging, heating, and homogenization were found.

The so-called "body" or "viscosity" of cream and ice cream mixtures has received much attention. Various methods have been used to measure this property and the results obtained have not always shown the expected relation to other properties of the cream or ice cream mixture. Many attempts have been made to correlate the freezing properties of ice cream mixtures with their viscosity. Some investigators believed that they found such a relation while others did not.

The results presented here show that what has usually been called the viscosity of cream or ice cream mixtures is made up of two factors which do not necessarily run parallel with each other. Investigators have taken some unknown relation between these two factors and have tried to relate it to the properties of the cream or ice cream mixture. In their investigations the results have been expressed as if the yield value were zero which this investigation shows is an unwarranted assumption in most cases. The character of this error can be easily shown with our data by assuming that the yield value is zero in all

cases and then calculating the results on that basis. As can readily be understood from an observation of the graphs which show a definite yield value, the so-called viscosity values will be higher the lower the shearing force at which the measurement is made. In order to bring out this effect more clearly and to

TABLE 4

A comparison of the plasticity constants with the "viscosity values" which would have been obtained at 1500, 1000, and 500 dynes shearing force, if the samples were considered to be viscous instead of plastic

SAMPLE ORDER NUMBER	DESCRIPTION	PLASTICITY CONSTANTS		"VISCOSITY" AT VARIOUS SHEARING FORCES		
		Yield value	Consistency	1500 dynes	1000 dynes	500 dynes
				cp.	*cp.*	*cp.*
1	Pasteurized 20.1 per cent fat cream fresh	8	3.21×10^{-2}	3.47	3.45	3.40
2	Pasteurized 32.7 per cent fat cream fresh	110	5.64	6.10	6.35	7.17
3	Pasteurized 34.8 per cent fat cream fresh	109	6.66	7.19	7.49	8.55
4	Pasteurized 32.7 per cent fat cream aged	124	6.67	7.24	7.60	8.85
5	Ice cream mixture no. 2	66	7.31	7.60	7.80	8.46
6	Ice cream mixture no. 4	93	10.7	11.2	11.8	13.0
7	Pasteurized 39.9 per cent fat cream fresh	120	12.4	13.4	14.1	16.4
8	Pasteurized 39.9 per cent fat cream aged	184	13.1	15.0	16.0	20.3
9	Ice cream mixture no. 5	150	18.1	19.0	20.1	24.3
10	Ice cream mixture no. 3	126	21.2	23.0	24.3	27.9
11	Ice cream mixture no. 1	264	20.4	24.5	27.4	41.8
12	Heated 44.9 per cent fat cream	226	26.4	31.0	34.0	47.9
13	Raw 44.9 per cent fat cream	262	37.9	45.8	51.2	79.2
14	Pasteurized 34.8 per cent fat cream homogenized	336	52.2	68.4	78.2	150.0

make the values comparable, the flow in cubic centimeters per second at the shearing forces of 1500, 1000, and 500 dynes was determined graphically and these values were substituted in equation (5). The results obtained are given in table 4. The data are arranged in ascending order of viscosity at 1500 dynes

shearing force. This procedure of recalculating the data gives the same kind of results as would be expected if the viscosity of the same set of samples was determined at the same time, in three different viscometers of widely varying shearing forces. The comparison made here is perhaps a little more justified since the various values were all obtained with the same instrument operated with different shearing forces.

The results in table 4, with the exception of sample 1, show that if the samples are assumed to be truly viscous the viscosity in centipoise is higher the lower the shearing force applied. In some cases the differences are quite large, for example sample 14 gave a viscosity of 68 centipoises at 1500 dynes and 150 centipoises at 500 dynes, or an increase of 120 per cent. The differences would be greater if a shearing force still lower had been used. Sample number 14 is about 20 times as viscous as sample number 1 at 1500 dynes and 44 times as viscous at 500 dynes.

The difficulties in finding a relation between the viscosity of ice cream mixtures and their freezing properties is shown by comparing samples 10 and 11. At 1500 dynes sample 11 is 6 per cent more viscous than sample 10; at 500 dynes it is 46 per cent more viscous, and the consistency of sample 10 is about 5 per cent greater than sample 11. Furthermore the samples are not all arranged in the same order of increasing viscosity as a comparison of samples 3, 4, and 5 shows.

While much useful and valuable information may result from single "viscosity" determinations yet, these few considerations indicate the difficulties which may arise in finding a simple relation between a single viscosity measurement and the other properties of cream or ice cream mixtures. Considerable time is required to determine the pressure-flow relationship for a single sample. This is a great handicap in routine testing. On the other hand the so-called viscosity results obtained at some single unknown shear gradient may be subject to great difficulties of interpretation since another viscometer might possibly arrange some of the samples in a different order of increasing viscosity and might produce widely different spreads between the results obtained. The results presented by Morten-

sen (10) bring out this last point. He gives the relative viscosity of raw 35 per cent fat cream as 1.57, with water as 1.00, a value considerably lower than the average value obtained by Kobler (6) for whole milk. The relative viscosity of the ice cream mixtures which Mortensen investigated ranged from 1.22 to 2.12.

Another difficulty enters in the study of suspensions. The viscosity-concentration relationship studied at a single shearing force may be such that if a series of samples is arranged in the order of increasing viscosity at one concentration, the order may be quite different at another concentration. Sharp and Gortner (12) have shown this to be true with flour-in-water suspensions. The results obtained by Leighton and Williams (7) (8) indicate that it might also be true with ice cream mixtures. Leighton and Williams (8) give calculations of the viscosity of the unfrozen part of the ice cream mixture during the freezing process. Their results on the effect of temperature lead one to suspect that occasionally at least one might find samples which would arrange themselves in one order of increasing viscosity at 0°C. and in an entirely different order at the lower temperatures where the actual freezing is taking place. The fact that the presence of ice in the mixture increases the viscosity or really the plasticity of the mixture tremendously must also be borne in mind.

The work of Leighton and Williams and the results presented here indicate that if a thorough study of the "viscosity" of ice cream mixtures in relation to their other properties is to be made, the rate of flow should be determined at various pressures for each of several temperatures and several concentrations. Adopting the terminology of Leighton and Williams (7), they should also be studied in connection with their apparent (before agitation) and basic (after vigorous agitation) plasticity. This would be a considerable task.

SUMMARY

1. The yield value and consistency of cream increased with fat content, aging, and homogenization and decreased by heating.

2. Ice cream mixtures of average composition were shown to vary considerably in yield value and consistency.

3. Some of the difficulties which may arise if plastic substances are treated as viscous were shown.

4. Attention is called to some of the difficulties in relating the "viscosity" of ice cream mixtures to their freezing properties.

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A STUDY OF THE "COMMON WHITE" YEASTS FOUND IN DAIRY PRODUCTS*

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At the present time a great deal of investigation is centered on the microflora of dairy products. At first investigators put much emphasis on the bacteria alone, but now attention has been turned to the yeast content of these products. Yeasts are often mentioned in reporting the microflora of dairy products without reference to any particular species or types. It was the purpose of this study to describe more in detail the so-called "common white" yeasts found in dairy products. These include the yeasts that produce typical, regularly circular, whitish, glistening, convex colonies with an entire edge and do not ferment lactose. These yeasts are distinguished from the chromogenic type by their lack of color and from the mycoderma and rapid liquifiers by the type of colony. The colonies of the "common whites" are much the same as those of the lactose fermenters, but the production of acid and gas in milk by the latter serves to readily distinguish the two. In addition to the study of the description some emphasis was put on the classification of the "common whites" and special note as to whether they might be of any importance in the normal processes or changes that take place in dairy products.

Hammer (1) suggested the name of Torula lactis-condensi for these yeasts in his studies on formation of gas in sweetened condensed milk. Cordes (2) isolated a number of this type of yeast in his study of yeast forms present in milk and milk products. Cordes and Hammer (3) refer to the "common white" yeasts as group IV, in studying the yeasts found in dairy products. They also state that it is a catchall group which further study will be certain to divide up on a more definite basis.

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Samples of milk, butter, cream, and allied dairy products were plated with whey agar, using 1 cc. of 2 per cent solution tartaric acid to keep down the bacteria. At times difficulty was encountered on account of excessive mold growth on the plates, but this was remedied by using whey agar containing 4 per cent NaCl. After forty-eight hours, at room temperature, the plates were examined for yeast colonies. The low power microscope was used to identify the colonies.

Purification was accomplished by repeated plating. After purification the yeasts were studied in much the same way that bacteria from dairy products are studied. Spore production was determined by staining whey agar cultures one to seven months old. Whey gelatin stab cultures were also stained for spores. Acid and gas production was studied in milk and 2 per cent sugar bouillons "Giant" colonies were also observed.

One hundred sixty cultures from cream, butter, soft cheese, milk and other minor sources were studied. The morphology of the cultures was given rather careful study, but it was very difficult to divide the yeasts definitely on this basis, because there was so much variation in form and size of cells. Quite definite results were obtained by different growth temperatures. Some of the cultures grew well both at room temperature and at 37°C., other cultures grew well at room temperature but poorly or not at all at 37°C. Three very distinct changes were brought about by inoculating into litmus milk, which were of much value in dividing the organisms into groups:

1. Sweet curdling of the litmus milk in ten to fifteen days, and a partial digestion of the casein in about thirty days.
2. Producing alkalinity in litmus milk in ten to fifteen days, the alkalinity increasing with age.
3. No change in litmus milk. Even after thirty days the litmus milk would appear the same as at the time of inoculation, yet slides made from the litmus milk cultures revealed that cells were present.

Chiefly on the basis of morphology, growth temperatures, and the action of litmus milk, the author concluded that the organisms

naturally divided into four types. Other characteristics which were not so outstanding seemed to substantiate this division.

It is very evident that "common white" yeasts are very numerous in dairy products. This type of yeast can be isolated from many different types of dairy products.

This type of yeast is also very resistant. The entire hundred and sixty cultures were left in the refrigerator for a period of over ten months at a temperature that varied from 0° to 10°C. After this storage period the cultures were still alive.

Spore formation did not seem to be very prevalent and seemed to depend to a large extent on the conditions under which the cultures were kept.

There is one characteristic of the "common white" yeasts that seems to be constant and that is the yellow coloration when the colonies become contaminated with Aspergillus niger. This yellow pigment formation was thought to be a possible differentiation of the groups; however, this proved to be a general characteristic and did not serve as a guide to division.

I. Type A. Large, rapid growing oval or elliptical yeast which grew well at both room temperature and 37°C. Sweet curdled litmus milk in ten to fifteen days, and partly digested the casein in thirty days.

II. Type B. Large, rapid growing, oval yeast with some elongated cells, which grew well at room temperature and very poorly, or not at all at 37°C. Produced no change in litmus milk.

III. Type C. Medium sized, rapid growing, oval yeast with some elongated cells, which grew both at room temperature and 37°C. Produced alkalinity in litmus milk in ten to fifteen days, the alkalinity increasing with age.

IV. Type D. Medium sized oval cells with some cells nearly round. No growth at 37°C. Produced alkalinity in litmus milk in ten to fifteen days, the alkalinity increasing with age.

The action of the "common white" yeasts is slow. It is concluded from this and their general behavior that they do not have much influence on the changes brought about in dairy products by microörganisms.

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THE INFLUENCE OF THE pH OF AGAR MEDIA UPON THE BACTERIAL COUNTS OF RAW AND PASTEURIZED MILK*

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A study of this subject was suggested by a statement in the American Public Health Association's publication entitled, "Standard Methods of Milk Analysis" (fourth edition, 1923, page 5) which is as follows:

> A medium consisting of the above ingredients, including a suitable peptone, ordinarily has a reaction between pH = 6.2 and 7.0. If within these limits, the reaction requires no adjustment for milk analysis. The most desirable reaction is about pH = 6.5 to 6.6, but any reaction between pH = 6.2 and 7.0 is allowable. No change in reaction should be made without carefully determining the H-ion concentration of the finished medium by the method described below.

The specific problems, therefore, are, first, to find whether or not there is a material variation in bacterial counts when beef extract agar is employed with the following pH values—6.2, 6.4, 6.6, 6.8, and 7.0—for plating raw milk. Second, to find the most satisfactory pH value when beef extract agar is employed with the following pH values—6.2, 6.4, 6.6, 6.8, and 7.0—for plating pasteurized milk.

The raw milk samples were taken from milk shipped to the University of Maryland Dairy. The individual shippers were selected because of their sanitary methods. Samples were taken both from the milk of individual shippers and from the pooled milk of several shippers.

A portion of the pasteurized milk samples were collected just after the milk had been cooled, others were taken from milk which had been held in the ice box for five or six hours at temperatures varying from 34° to 40°F., while still other samples

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were taken from milk which had been carried on trucks over a milk route, and returned to the refrigerator.

The directions given in the before mentioned edition of Standard Methods of Milk Analysis were followed in the preparation of the media, plating, incubation, and in counting.

The experiment was started about the first of October and ran through the month of March. One hundred samples each of raw and pasteurized milk were examined. Five samples of raw and five of pasteurized milk were plated, in dilutions of 1:100, 1:1,000, and 1:10,000 each week.

Agar of pH values of 6.2, 6.4, 6.6, 6.8, and 7.0 were prepared every two weeks for plating this milk. The reaction was determined by the colorimetric method, using the LaMotte 3 B. set and following the procedure recommended by its manufacturers. The indicator, brom thymol blue, with a range of 6.0 to 7.6 was used. After the pH was determined, the agar was adjusted to the desired point.

TABLE 1

Influence of pH of agar media upon bacterial counts of milk

pH	RAW MILK		PASTEURIZED MILK	
	Average count, 99 samples	Mean variation	Average count, 100 samples	Mean variation
6.2	51,455	±6,824	30,342	±3,575
6.4	52,797	±7,911	31,293	±3,585
6.6	53,103	±7,621	30,634	±3,413
6.8	53,210	±8,491	30,396	±3,501
7.0	50,739	±6,608	29,857	±3,521

It is well known that media does not always show an increase in titrable acidity after autoclaving. To correct for such a change, a sample was autoclaved to determine the drop and enough alkali was added to make up for any deficiency. Most of the media made showed an initial titration of from 6.0 to 6.2. The media was then tubed and autoclaved at 15 pounds pressure for twenty minutes, cooled and placed in the ice box until used.

Table 1 shows the data.

The results obtained by plating 99 samples of raw milk showed but little variation in counts. The medium having a hydrogen ion concentration of 6.8 gave the highest average bacterial count. The other media ranged as follows: 6.6, 6.4, 6.2, and 7.0.

The results obtained by comparing the results of one hundred samples of pasteurized milk plated on beef extract agar, titrated to the before mentioned pH's, like raw milk, showed but little variation. The medium having a hydrogen ion concentration of 6.4 gave the highest average bacterial count, the others ranged in the following order: 6.6, 6.8, 7.0, and 6.2.

CONCLUSION

After a study of the available literature, together with the foregoing data, the following conclusion would seem warranted:

If the bacterial counts of raw and pasteurized milk plated on beef extract agar are significantly affected by the hydrogen ion concentration of the media when ranging between 6.2 and 7.0, the 199 samples are not sufficient to show this significance.

THE BACTERIAL CONTENT OF ORANGE SHERBET*

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A great deal has been said and written about the increase in the consumption of ice cream during the past two decades; less publicity, however, has been given to the increasing popularity of sherbets and ices. It is quite generally agreed that the increase in demand has been phenomenal, but there are no statistics available in this country which show the amount of these products manufactured each year.

It has been claimed that the increased demand for the lighter frozen desserts has been due to the modern fad of dieting, nevertheless, it must be recognized that there has been a marked improvement in the flavor and texture of these delicacies which has elicted public attention and favor. Likewise, it must be recognized that development of the public confidence in ice cream as a safe, wholesome, and sanitary food has had a marked effect on the sale of frozen desserts in general.

In view of the fact that ices and sherbets for the most part are made from ingredients usually considered relatively free from bacteria, very little attention has been paid to their sanitary control. Ices usually contain water, sugar, gelatin, eggs, and either fresh or preserved fruits, whereas the sherbets have in addition a greater or less amount of ice cream mix or dairy products. One would expect the water, sugar, eggs, and fruit to contain very few bacteria. Most of the gelatin (1) now used by ice cream makers is likewise very low in bacterial count. The ice cream mix is the only ingredient that is likely to be uncertain in this respect. If the ice cream mix has been made from high grade raw products, properly pasteurized, and processed under controlled conditions, it should contain relatively few bacteria.

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The water used in compounding the sherbet or ice mix should be of known purity and should be tested at frequent intervals, especially if the water is from a private well. Sound, fresh fruits which have been properly washed should introduce relatively few bacteria, and preserved fruits are ordinarily practically sterile. Sugar which has been kept dry contains very few bacteria. Eggs are either sterile or contain such small numbers of bacteria that the quantitative contamination from this source is negligible.

From the foregoing statements it seems logical to assume that ice or sherbet mixes, which have been made from high grade products, would contain very few bacteria before processing. If processed in clean equipment and carefully aged at low temperatures, the bacterial content of the finished product should also be relatively low. If the bacterial content of a sherbet or a water ice is high, the most likely sources of contamination are, poorly washed utensils, improper aging temperature, or, in case of a sherbet, to the addition of ice cream mix or dairy products of poor quality.

On account of the harmless nature of the ingredients used, ices and sherbets are not likely to attract the interest of the public health official. However, there would be no easier way for an inspector to detect insanitary plant methods, especially improperly washed equipment, than by means of a bacterial analysis of a water ice. The relatively low bacterial content of a water ice or sherbet enables the detection of equipment contamination of lesser magnitude than would be possible with other products, such as ice cream or milk. For example, bacterial analyses on sherbets or ices before and after freezing afford the plant manager or the inspector an excellent method of checking the efficiency of the cleaning methods in use on the freezer, and at the same time, give some measure of the general sanitary quality of the product.

The question is frequently raised as to whether or not it is necessary to pasteurize the water ice or sherbet mix. In general it may be said that the pre-heating of any food, where it is feasible, is always desirable. The high acidity of some mixes probably destroys certain pathogens, however, this factor is

neither sufficiently constant nor reliable to use as a substitute for pasteurization. On the other hand, it has been suggested that there is no more necessity for pasteurizing the water ice or sherbet mix than for similarly treating salad dressing or other such foods ordinarily prepared and consumed without previous cooking. It must be granted that even though the ingredients of water ices and sherbets are not so likely to introduce pathogenic organisms, these products are subjected to the same possibilities of human contact during processing as is the ice cream mix. Human contact with uncooked foods is always a potential source of danger and should be avoided wherever possible. Although the pasteurization of water ice and sherbet mixes may not be quite so imperative as it is for the ice cream mix, the process is

TABLE 1

The bacterial counts on 21 samples of orange sherbet

Plate count per gram of sherbet		
80	2,000	20,000
180	2,100	24,000
600	3,000	31,000
700	3,400	55,000
850	3,800	64,000
1,400	7,000	180,000
1,600	15,000	1,100,000

sufficiently advantageous to more than justify its adoption. In the plants where pasteurization of the water ice and sherbet mixes is not practiced, special precautions should be taken to prevent human contact with the product and to insure the sanitary quality of the ice cream mix, dairy products, and water used.

Bacterial analyses of sherbets and ices are very seldom reported in the literature, either because of lack of interest or because it is not a common practice to make such determinations. Hammer (2) reported the analysis of 17 water sherbets which ranged from 6 to 7800 bacteria per cubic centimeter. In connection with the 1928 session of the Kansas Ice Cream Scoring Contest, as described by Fay and Martin (3), opportunity was afforded to

make bacterial analyses of 21 samples of orange sherbet. These samples were representative of the product sold in the middle west. Most of them were manufactured in Kansas, some in Missouri, and some in Nebraska.

The bacterial analyses were made on a gravimetric basis, as described by the American Dairy Science committee report on Bacteriological Methods for Examining Ice Cream (4). The difficulty encountered in expelling the air from some of the sherbets made it imperative to use the gravimetric in preference to the volumetric method. A 10-gram sample was used in order to minimize the error in weighing which resulted from the presence of relatively large pieces of fruit. Plain agar, prepared according to the standard methods of the American Public Health Association, was used, and the plates were incubated forty-eight hours at 37°C.

The results of the 21 analyses are given in table 1, and express the number of colonies per gram of sherbet.

A summary of the results shows that of the 21 samples:

10 per cent contained less than	200 bacteria per gram
24 per cent contained less than	1,000 bacteria per gram
57 per cent contained less than	5,000 bacteria per gram
76 per cent contained less than	25,000 bacteria per gram
90 per cent contained less than	100,000 bacteria per gram
10 per cent contained over	100,000 bacteria per gram

In view of the limited information at hand, it would not be feasible to suggest any definite figure as a basis for judging the bacterial content of sherbets.

The bacterial counts on these sherbets are presented solely to bring to light the possible value of bacterial analysis of sherbets and water ices as a means of detecting faulty plant methods, as well as a criterion of sanitary quality. The use of poor ingredients or improperly washed equipment is practically certain to be detected in a bacterial analysis of the finished product. The fact that a food product contains only the best grade of ingredients, and that it has been produced in clean equipment with scrupulous care is one of the most important criteria of quality. The ice cream maker who is attempting to cater to the

increasing public demand for foods of high sanitary quality may very profitably give heed to the results of bacterial analysis of his water ices and sherbets.

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THE VOLUME OF THE CREAM LAYERS FORMING ON HOLSTEIN AND JERSEY MILK*

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Prior to the introduction of the centrifugal cream separator the problem of the creaming ability of milk from the various breeds of cattle was of special economic importance. The numerous investigations of about twenty-five years ago compared the creaming properties of milk from the standpoint of the richness of the cream and the completeness and rapidity with which the fat in the skimmilk rose to the cream layer. These early investigations showed the milk of the Channel Island breeds to possess the best creaming properties. In recent years the fat losses in skimmilk and the test of cream obtained by gravity skimming have become insignificant but the depth of the cream layer which forms on milk is important. At the present time the old meaning of the creaming ability of milk is almost obsolete and the term will be used in this article in its newer meaning to designate the ability of milk to form a cream layer with a distinct visible line or division between the cream and skimmilk.

Recently, Palmer and Anderson (1) separated rich creams from Jersey-Guernsey and Holstein milk which were standardized with Jersey-Guernsey and Holstein skimmilk to give milks containing 3.5 per cent of fat. The Jersey-Guernsey milk gave deeper cream layers than Holstein milk which was found to be associated with the properties of the Jersey-Guernsey skimmilk rather than with the cream.

It was the intention of this investigation to determine the depth of cream layers which form upon fresh, normal milk produced by registered Holstein and Jersey cattle. The results of this study are not comparable with those in the reference just given because of differences due to standardization and aging of the products.

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The variation in the cream layer volumes caused by these factors will be considered in subsequent papers.

In view of various methods of setting milk for cream layer formation it was essential that a standard procedure should be followed for handling the milk, setting the tubes, and making the readings. A large number of tests were made using 100 cc. graduated cylinders, and test tubes as recommended by Harding (2). The results secured by the use of both containers were identical. The cylinders were more easily read, but less convenient to handle, and more expensive. For these reasons 100 cc. cylinders were used, or tubes of such a size that they could be fitted into a graduated cylinder in which position they were filled with milk and later re-inserted for reading. A slight insignificant error in the total amount of milk used in each test was introduced by the latter method.

The milk used in each test was the complete milking of one cow which was cooled to approximately 10°C. (50°F.) by a tubular surface cooler. Within a minute or two after milking and cooling, 100 cc. of each sample of milk in duplicate tubes were placed in a water bath at 2.8° to 4.4°C. (37° to 40°F.). The length of the cream layer was measured at two-, four-, and twenty-four-hour intervals. Preliminary tests showed that warm milk placed directly into tubes not in contact with other tubes was cooled to the temperature of the surrounding water in less than thirty minutes and the cream layers were comparable to those which developed on tubular cooled milk. It was also found that the temperature of the milk during the creaming period should be maintained within the specified temperatures to secure comparable results.

The samples of Holstein milk were secured from 50 registered cows maintained by L. A. Colton at a farm near the Experiment Station. The samples of Jersey milk were taken principally from the station herd of 26 cows and to a smaller extent from 22 registered Jerseys owned by C. R. Pontius of MacDougall. A total of 627 Holstein and 900 Jersey samples of milk were used in the tests. The milk was set in the milk room at the barn for cream rising observations. Records were kept of the age and health of the

cows, milk production, and stage of lactation period so that this information would be available. Most of the data were secured between June 1, 1926, and July 1, 1927.

The variations in the percentages of the fat in the milk could not be adjusted to a uniform standard without the possible introduction of some unknown variant in the creaming properties. For this reason comparable expression of results was obtained by dividing the percentage which the volume of the cream layer was of the total volume of milk by the percentage of fat which the milk contained. This result or ratio represents the percentage volume of cream for each per cent of fat which the milk contained. The majority of Holstein samples contained 3.1 to 3.7 per cent fat, whereas the Jersey milk tested from 5.0 to 7.0 per cent.

TABLE 1

The changes in the cream layer, calculated for 1 per cent of fat, on Jersey and Holstein milk due to time held at 40°F.

KIND OF MILK	NUMBER OF CASES	AVERAGE FAT PERCENTAGE	2-HOUR PERIOD		4-HOUR PERIOD		24-HOUR PERIOD	
			Cream layer	No layer	Cream layer	No layer	Cream layer	No layer
Jersey	282	5.9	5.54	67	4.76	40	4.11	13
Holstein	164	3.4	5.53	24	4.85	21	4.09	14

INFLUENCE OF HOLDING TIME ON CREAM LAYER VOLUME

Practical experience and several investigations have shown that milk should be stored at 1.7° to 4.4°C. (35° to 40°F.) to obtain the deepest cream layer. The time of reading the depth of the cream layers has been rather variable although twenty-four hours has been most generally used. In commercial practice shorter periods of time may be more important as milk is delivered to consumers within twenty-four hours or less. The cream layers were measured after two-, four-, and twenty-four-hour periods in this study but the twenty-four-hour results were used in most calculations.

In table 1, the two-, four-, and twenty-four-hour cream layer volumes calculated for 1 per cent of fat are given for 282 samples of Jersey and 164 samples of Holstein milk. A large portion of

these samples failed to show definite cream lines within the two- and four-hour periods which was especially pronounced for Jersey milk. The volume of the cream layers formed were very similar for the milk of the two breeds, a result that will be considered again. The depth of the cream layers decreased with time but they had

TABLE 2

The creaming ability of Holstein and Jersey milk presented on a comparable basis by calculating the normal cream layer for 1 per cent of fat

CREAM LAYER FOR 1 PER CENT OF FAT	HOLSTEIN MILK FROM 47 COWS		JERSEY MILK FROM 47 COWS	
	Frequency	Percentage frequency	Frequency	Percentage frequency
No layer	14*	0	13*	0
2.00-2.99	8*	0	1*	0
3.00-3.19	0	0	9	3.2
3.20-3.39	5	2.5	15	5.3
3.40-3.59	9	4.8	27	9.6
3.60-3.79	22	11.9	30	10.6
3.80-3.99	28	15.3	34	12.1
4.00-4.19	35	18.9	43	15.3
4.20-4.39	36	19.4	33	11.8
4.40-4.59	26	14.1	31	11.0
4.60-4.79	16	8.6	23	8.2
4.80-4.99	8	4.3	12	4.3
5.00-5.19	0	0	7	2.5
5.20-5.39	2*		5	1.8
5.40-5.59	0		5	1.8
5.60-5.79	0		1	0.4
5.80-5.99	0		3	1.0
6.00-6.19	0		2	0.7
6.20-6.39	0		0	0
†Mean cream layer.....	4.09 ±0.02		4.11 ±0.03	
Standard deviation.....	0.55 ±0.02		0.64 ±0.02	

* Samples not used in calculation of mean or percentage frequencies.

† The means, standard deviations, and their probable errors were calculated from the original data which yield slightly different values than those given by the data as grouped in this table.

shrunk to a minimum within the twenty-four-hour period. The shrinking of the cream layers formed on raw milk has been observed by Hammer (3) and others. It is evident that in experimental studies of creaming that observations should be made at various intervals.

CREAMING ABILITY OF HOLSTEIN AND JERSEY MILK

The cream layer volumes which were present on Jersey and Holstein milk after twenty-four hours standing at 37° to 40°F. are summarized in table 2 and graphically represented in figure 1. The data are arranged according to frequencies of increasing cream layer volumes. The mean cream layer volume for one per cent of fat in Holstein milk was 4.09 ±0.02 and for Jersey Milk 4.11

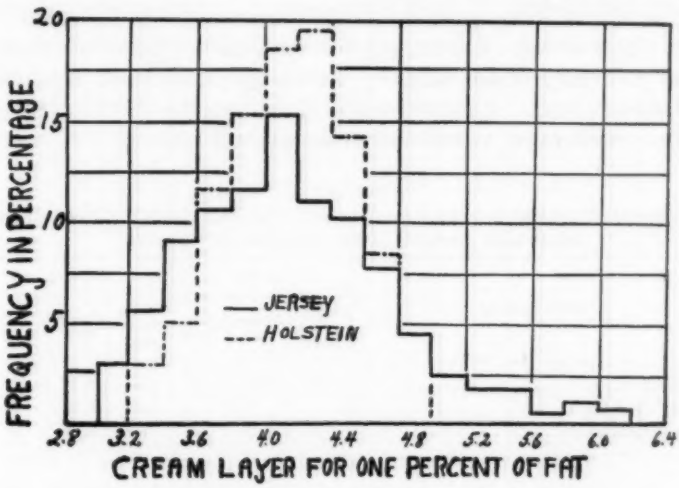

FIG. 1. THE CREAMING ABILITY OF HOLSTEIN AND JERSEY MILK SHOWN AS THE FREQUENCY, EXPRESSED IN PERCENTAGES, WITH WHICH THESE MILKS GAVE CREAM LAYERS OF VARYING VOLUMES PER 1 PER CENT OF FAT

±0.03. In view of the very small probable errors of these means and the slight difference between the cream layer volumes formed on these two kinds of milk it is clear that there is no difference between the cream volumes. It should be noted that the cream layer volume to be expected on any given sample of fresh Holstein or Jersey milk is rather variable since the standard deviations of the mean volumes are 0.55 ±0.02 and 0.64 ±0.02, respectively.

These results, being contradictory to established belief, warrant

a few words concerning their accuracy. There can be no question about the similarity of the cream layer volumes for one per cent of fat which formed on either Holstein or Jersey milk used in these trials. The number of cows and samples of milk were sufficient to justify conclusions. The principal source of possible error would be that the 47 cows of each breed whose milks were used in these particular tests were not typical for the breeds due to local conditions of some character.

The milk of Jersey cows is not only more variable in the rapidity with which a cream line formed, but the final volume of the cream layer includes a wider range of percentages than does the milk of Holstein cows. Figure 1 clearly illustrates the closer grouping of the cream layer volumes of Holstein milk around the mean.

TABLE 3

The cream layer volume, calculated for 1 per cent of fat, which formed on Holstein and Jersey milk produced in the winter and in the spring

KIND OF MILK	WINTER			SPRING		
	Number of cases	Cream layer	No layer	Number of cases	Cream layer	No layer
Holstein	84	4.05	0	67	4.12	11
Jersey (Pontius)				56	3.97	9
Jersey (Station)	113	3.86	3	95	4.30	6

The standard deviations, as given in table 2, confirm the graph, for the standard deviation for the cream layer volume of Jersey milk is 0.09 greater than that of Holstein milk.

It was recognized at the beginning of this study that factors other than the breed of cattle may influence the normal creaming ability of the milk as it is drawn from the cow. Records were kept of the age and health of the cow, regularity of milking periods, amount of milk given and stage of the lactation period. In a few isolated cases the results obtained with certain samples of milk were discarded due to these causes since the samples were taken with a knowledge of the possibility of discrepancies due to them. The data were grouped on the basis of the season of the year in which the milk was produced so that the creaming ability

of milk produced in the winter on dry feeds, including silage and mangels, could be compared with the creaming properties of milk produced on pasture grass. The summarized figures for Jersey and Holstein milk produced under these conditions are given in table 3.

TABLE 4

The creaming ability of milk from individual cows presented on a comparable basis by calculating the normal cream layer for 1 per cent of fat

COW NUMBER	HOLSTEIN HERD					JERSEY HERD (PONTIUS)					JERSEY HERD (STATION)				
	Number of trials	Cream layer for 1 per cent of fat				Number of trials	Cream layer for 1 per cent of fat				Number of trials	Cream layer for 1 per cent of fat			
		Minimum	Maximum	Average	No layer		Minimum	Maximum	Average	No layer		Minimum	Maximum	Average	No layer
1	6	3.3	3.9	3.6	0	4	3.7	5.8	4.5	0	13	3.3	4.8	4.0	0
2	4	4.1	4.5	4.3	0	4	3.3	4.6	3.8	0	13	3.3	4.9	4.1	0
3	6	4.0	4.7	4.3	0	4	3.7	5.6	4.6	0	11	3.8	5.4	4.1	0
4	5	3.7	4.7	4.0	0	4	4.3	5.5	4.9	2	13	3.4	4.6	3.9	0
5	6	3.5	4.1	3.9	0	3	3.3	5.0	3.9	0	14	3.8	6.1	4.7	6
6	6	3.7	4.6	4.1	0	4	3.5	5.7	4.3	0	10	3.2	6.8	5.2	2
7	4	4.3	4.5	4.4	0	3	4.4	6.1	5.1	0	11	3.9	4.9	4.2	0
8	6	4.0	4.8	4.3	0	3	4.6	4.6	4.6	2	9	4.3	5.3	4.8	0
9	6	3.6	4.3	3.9	0	4	3.9	4.7	4.3	0	13	3.1	4.5	3.9	0
10	4	3.7	4.3	4.0	0	4	3.5	5.5	4.3	0	13	3.5	4.8	4.1	0
11	4	3.5	3.8	3.6	0	4	3.9	4.8	4.2	1	12	3.3	4.6	4.0	0
12	6	4.0	5.2	4.5	0	4	3.4	5.0	4.1	0	9	3.0	4.5	3.7	0
13	3	4.0	4.3	4.2	0	4	3.8	5.4	4.5	0	7	4.7	6.0	5.4	1
14	3	3.4	3.9	3.7	0	4	4.3	5.6	4.9	0	10	4.1	6.1	4.7	0
15	3	4.4	4.5	4.5	0	4	3.0	4.8	3.9	0	7	3.6	6.2	4.9	0
16	5	3.8	4.6	4.2	0	4	3.9	4.7	4.3	0	9	3.2	4.5	3.7	0
17	5	4.0	4.8	4.3	0	3	4.1	5.6	4.7	0	10	3.3	4.7	3.9	1
18	3	3.5	4.0	3.8	0	4	3.5	4.4	3.9	0	8	3.6	4.1	3.7	1
19	3	3.5	3.8	3.7	0	4	3.3	5.0	4.1	0	8	3.5	4.9	4.0	0
20	3	4.3	4.4	4.3	0	2	3.2	3.4	3.3	0	3	3.1	3.8	5.5	0

The grouping of the figures in this manner again illustrates the uniform mean cream layers, calculated for 1 per cent of fat in the milk, which form on Holstein and Jersey milk. The more uniform creaming of Holstein milk is also shown. There was no significant difference between the creaming properties of Holstein milk produced in the spring and winter months. The interpreta-

tion of the results secured with Jersey milk is difficult because there appears to be a difference between the creaming ability of milk produced by the station Jersey herd which is contradicted of the creaming properties of the spring milk produced by the

TABLE 5

The relationship of cream layer, per 1 per cent of fat, to specific gravity of milk

SPECIFIC GRAVITY OF MILK	PER CENT FAT	TOTAL SOLIDS CALCULATED	CREAM LAYER		
			2-hour period	4-hour period	24-hour period
1.0290	4.2	12.3	No layer	5.2	4.8
1.0300	5.0	13.5	7.4	5.4	4.2
1.0301	5.4	14.0	No layer	5.2	4.4
1.0303	5.5	14.2	5.1	3.2	2.9
1.0309	5.4	14.2	No layer	No layer	No layer
1.0313	5.7	14.6	No layer	4.9	4.2
1.0325	2.8	11.5	5.3	4.6	4.3
1.0325	5.6	14.8	5.4	5.0	3.9
1.0327	2.9	11.6	5.5	4.9	4.4
1.0327	5.0	14.2	No layer	7.2	5.2
1.0330	2.8	11.5	4.6	4.6	4.3
1.0331	6.0	15.5	No layer	5.1	4.3
1.0333	6.6	16.2	No layer	5.0	4.2
1.0335	6.2	15.8	No layer	4.5	4.0
1.0335	6.2	15.8	No layer	No layer	No layer
1.0339	4.8	14.2	5.2	5.0	4.2
1.0339	5.6	15.2	No layer	6.4	5.0
1.0340	4.0	13.3	6.2	5.2	4.7
1.0340	6.2	15.9	No layer	5.4	4.6
1.0340	6.5	16.3	No layer	No layer	No layer
1.0342	5.3	14.9	No layer	6.8	5.2
1.0348	4.7	14.3	5.9	5.7	4.7
1.0350	4.5	14.1	6.2	4.9	4.0
1.0350	4.8	14.5	No layer	4.6	3.9
1.0350	4.8	14.5	5.0	4.6	3.7
1.0350	4.8	14.5	5.4	5.0	3.7
1.0366	4.2	14.1	6.2	5.2	5.0
1.0370	4.2	14.3	6.2	5.7	5.2

other Jersey herd. Considered as a whole, these cream layers which formed on 415 samples of milk are not sufficient in number to show a difference between the creaming ability of milk produced in the winter or spring.

These data were also arranged and studied to show a relationship between the fat content of milk and the cream layer volume per 1 per cent of fat. When the milks of both breeds were considered together there were sufficient samples testing between 4 and 5 per cent to give a complete range of tests from 3 to 7 per cent. There appeared to be no other relationship between the percentage of fat and the cream layer volume than the value already given of 4.1 per cent of cream layer for each 1 per cent of fat in the milk. The data are not presented according to richness of the milk since no difference in the cream layer volumes per 1 per cent of fat were observed in table 2 comparing Holstein and Jersey milk.

A number of specific gravity determinations were made on samples of normal milk by the Westphal Balance. The results of these determinations, together with the cream layers which formed on the milk after two, four, and twenty-four hours, are given in table 5. The specific gravity of the milk was not associated with the creaming ability of the milk, a conclusion which is in agreement with the work of Palmer, Hening, and Anderson (4), and others.

VARIATIONS IN CREAMING ABILITY OF MILK FROM INDIVIDUAL COWS

Hammer (3) found that the cream layer volumes for milk produced by 10 different cows varied from 4.9 to 7.4 for each 1 per cent of fat contained in the milk. Others have observed this same variation. The cream layer measurements made in this investigation permitted a more extensive study of this factor as a variant in the creaming ability of milk.

The data given in table 4 include cream layer measurements made from 2 to 14 milk samples taken from each cow. The milk from 60 cows was used in this comparison. The cows varied in age, production, and stage of lactation period. Not only does the creaming of the milk from one cow differ from that of another but in most instances the different samples of milk from the same cow vary widely. In a few cases a cow produced milk which, from day to day, possessed rather uniform creaming powers.

Expressed in another way, this study has shown that one cannot predict with accuracy the creaming ability of the milk of an individual cow from a knowledge of previous cream layer determinations on the milk. An exception must be made for cows that produced milk which gave no visible cream line in a large percentage of trials and for certain cows which gave milk that tended to give abnormally deep or shallow cream layers even though their depth varied from sample to sample.

SUMMARY

1. The comparative creaming properties of milk may vary according to the procedure followed in making the tests. For greatest uniformity and the exclusion of unknown factors the milk was set for creaming within a few minutes after milking in ice water at 2.8° to 4.4°C. The cream layers of different samples showed variations in the cream layer volume and the distinctness of the cream line after two or four hours which were less pronounced after twenty-four hours.

2. The mean depth of the cream layers forming on normal milk from Holstein and Jersey cows was directly proportional to the percentages of fat which they contained. The percentage which the cream layer represented of the total volume of milk was about 4.1 times the percentage of fat.

3. The variations in the cream layer volumes forming on individual samples of Jersey milk were greater than those for Holstein milk. This difference was especially noticeable after two and four hours of creaming.

4. The milk from an individual cow did not possess uniform creaming properties from milking to milking although there was a tendency for the milk from certain cows to give either large or small cream layer volumes or a high percentage of no layer cases.

5. The cream layer volumes on the samples of milk used in these tests were not affected by the season of the year in which the milk was produced. The milk from Holstein cows remained most uniform throughout the year.

6. Variations in specific gravity of milk were not related to changes in creaming properties.

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THE CONCENTRATED WATER SOLUBLE FRACTION OF MILK AS A SOURCE OF VITAMIN B*

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Although milk as a source of the vitamin B complex is not particularly noteworthy, its quota of this accessory food factor is sufficiently adequate to warrant the assumption that a concentrate of the water soluble fraction would contain an appreciably greater quantity of this vitamin than is found in the natural milk or in any of its concentrated commercial forms.

Such a concentrate has been prepared under commercial and semi-commercial conditions. Briefly, the procedure involves the successive removal from the milk, of the fat or cream by skimming, the casein by heating with a suitable precipitant, the lactalbumin by coagulating with heat, a considerable proportion of the insoluble calcium phosphate, and by repeated crystallizations, the greater part of the milk sugar. The residual liquor or serum may be concentrated to any desired degree, as for example to a viscous fluid, containing about 40 per cent solids, a thick paste containing 65 to 80 per cent solids, or even to dryness. Since the method of preparation is carried out with the object of utilizing the obtainable water soluble constituents of milk, minus those minor increments lost by adsorption and occlusion and minus a large part of the lactose, the final serum or liquor contains the so-called soluble nitrogen extractives, the water soluble milk minerals, a certain proportion of milk sugar and the vitamin B of the original fluid milk, concentrated to a high degree.

The composition of the dry solids of the water soluble fraction, prepared as described, in comparison with the composition of the dry solids of milk is shown in table 1. The alteration of the mineral balance is shown by comparing the ash constituents of natural milk with those of the water soluble fraction as in table 2.

* Received for publication June 19, 1928.

TABLE 1

The composition of milk solids and the solids of the water soluble fraction

	MILK SOLIDS (DRY BASIS)		COMMERCIALLY PREPARED WATER SOLUBLE FRACTION (DRY BASIS)	
	per cent		*per cent*	
Fat	27.25		00.00	
Ash	6.25		28.37	
Lactose	39.00		57.27	
Total nitrogen	4.31		2.15	
Protein nitrogen		4.04		0.67
Non-protein nitrogen		0.27		1.48
Ammonia nitrogen				0.19

TABLE 2

The composition of the ash constituents of milk solids and the solids of the water soluble fraction

	MILK SOLIDS (ASH CONSTITUENTS)	COMMERCIALLY PREPARED WATER SOLUBLE FRACTION (ASH CONSTITUENTS)
	per cent	*per cent*
P_2O_5	26.87	4.28
CaO	22.28	8.31
MgO	2.48	2.11
K_2O	27.83	34.89
Na_2O	6.64	15.27
Cl	13.28	41.08
SO_3	2.25	1.44
Undetermined	2.02	2.10
Total	103.65	109.48
0 = Cl	3.65	9.48
	100.00	100.00

VITAMIN B CONTENT OF THE SOLIDS OF THE WATER SOLUBLE FRACTION

Since the particular object of this study was to determine the relative vitamin B content of the solids of the water soluble fraction of milk, prepared as already indicated, numerous feeding experiments have been conducted in parallel with variable quanti-

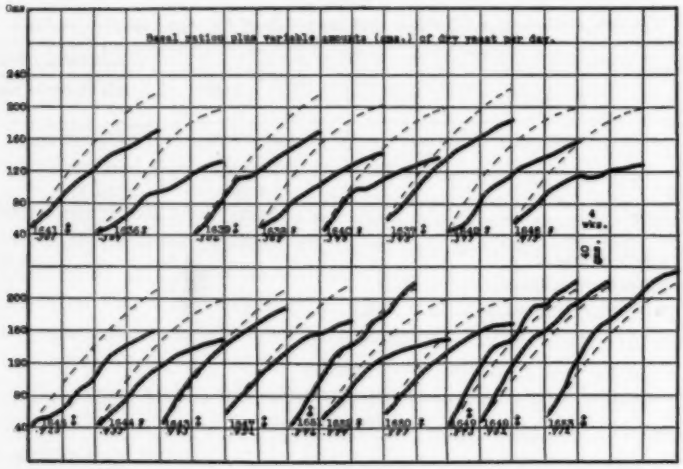


CHART 1. DRY YEAST AS A SOURCE OF VITAMIN B

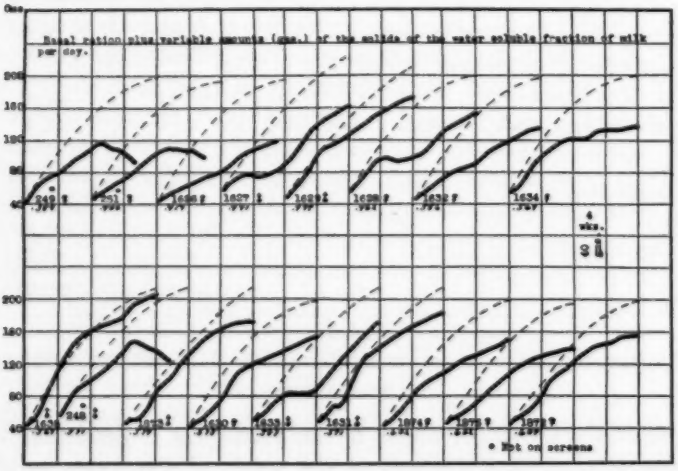


CHART 2. THE SOLIDS OF THE WATER SOLUBLE FRACTION OF MILK AS A SOURCE OF VITAMIN B

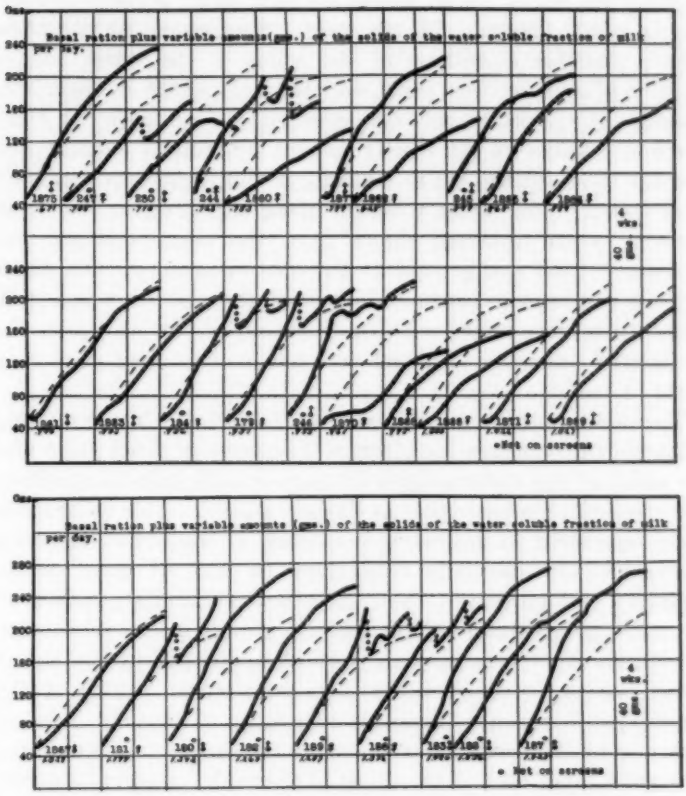

Basal ration plus variable amounts (gms.) of the solids of the water soluble fraction of milk per day.
4 wks.
Basal ration plus variable amounts (gms.) of the solids of the water soluble fraction of milk per day.
4 wks.

CHART 2 (*continued*). THE SOLIDS OF THE WATER SOLUBLE FRACTION OF MILK AS A SOURCE OF VITAMIN B

ties of dry yeast. White rats reared under standardized conditions at our own laboratory were used. At the age of twenty-eight to thirty days, young animals weighing from 40 to 50 grams were placed in individual cages with screened bottoms, (see exceptions noted on accompanying charts). The following basal ration was fed: casein purified by acetic acid, 18 parts; salt

mixture no. 40,[1] 4 parts; powdered agar agar, 2 parts; butterfat, 5 parts; dextrin, 71 per cent. Supplementing the basal ration, variable quantities of the solids of the water soluble fraction of milk, or dried yeast were fed daily as the sole source of vitamin B. Dried yeast was selected as the basis of comparison, because of its widely known vitamin B content and because of its general use as a carrier of this important accessory food factor. The yeast used for these experiments was fresh brewers' yeast obtained direct from the manufacturers, immediately dried and ground at our own laboratory. For those animals not kept in screened bottom cages, the yeast and milk concentrate were mixed with the basal ration; for all others the designated amounts were fed separate in the form of a thin paste or pellet.

The accompanying growth curves (charts 1 and 2) adequately illustrate the comparative vitamin B potency of dried yeast and the solids of the water soluble fraction of milk with composition and method of preparation as already stated. The evidence is fairly consistent in indicating a vitamin B content of the milk concentrate substantially equivalent to that of dried yeast. Figures 1 to 5 on plate 1 show the effect of variable amounts of the solids of the water soluble fraction of milk as the sole source of vitamin B. These animals were photographed at the age of eleven weeks, or seven weeks after continuous feeding of the experimental ration. Complete adequacy of vitamin B is illustrated by the condition of the animal in figure 1. This animal received 8.7 per cent of the solids of the milk concentrate mixed with the basal ration. During the first seven weeks of the experimental feeding period the average daily intake of the milk concentrate solids was 1.019 grams. Lesser amounts were furnished the animals shown in figures 2, 3 and 4, the average daily intake amounting to 0.359, 0.063 and 0.067 gram respectively. The animal shown in figure 5 received no vitamin B from any source whatsoever, and typifies the results of such complete deficiency.

[1] Steenbock, H., and Nelson, E. M., Jour. Biol. Chem., lvi, p. 362, 1923.

CONCLUSIONS

Data are given which show that the solids of the water soluble fraction of milk, when properly prepared and concentrated, are highly potent in vitamin B content and compare favorably with dried yeast as a source of the growth promoting and antineuritic factor.

PLATE 1

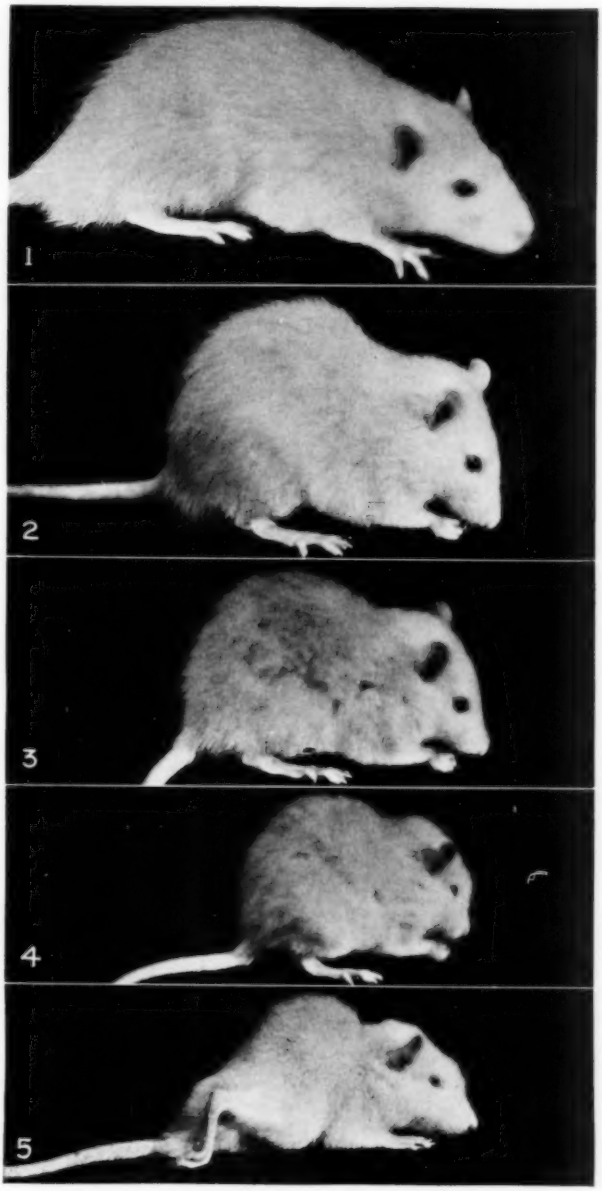
FIG. 1. Results obtained from feeding 1.019 grams daily for seven weeks of the solids of the concentrated water soluble fraction of milk as the sole source of vitamin B.

FIG. 2. Results obtained from feeding 0.359 gram daily for seven weeks of the solids of the concentrated water soluble fraction of milk as the sole source of vitamin B.

FIG. 3. Results obtained from feeding 0.063 gram daily for seven weeks of the solids of the concentrated water soluble fraction of milk as the sole source of vitamin B.

FIG. 4. Results obtained from feeding 0.067 gram daily for seven weeks of the solids of the concentrated water soluble fraction of milk as the sole source of vitamin B.

FIG. 5. Results obtained from feeding basal ration only without any vitamin B whatsoever for seven weeks.


1
2
3
4
5

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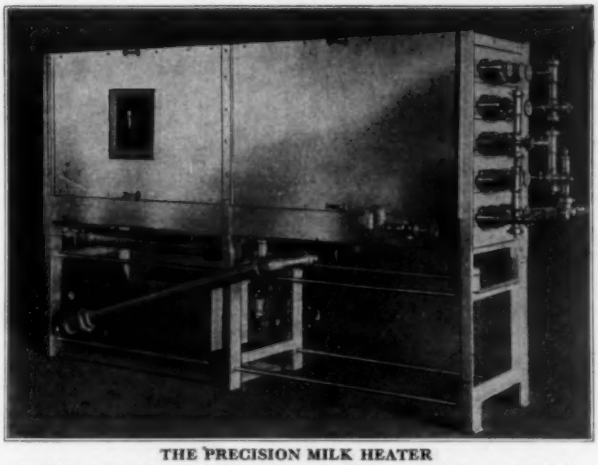

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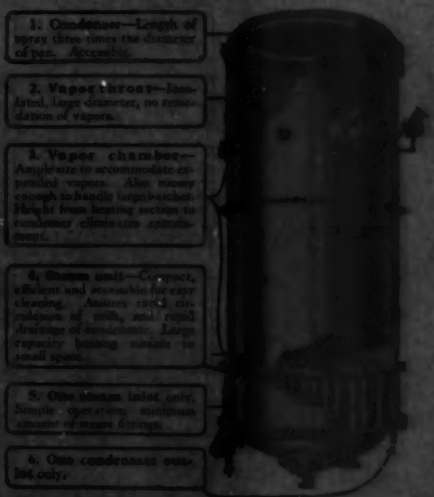
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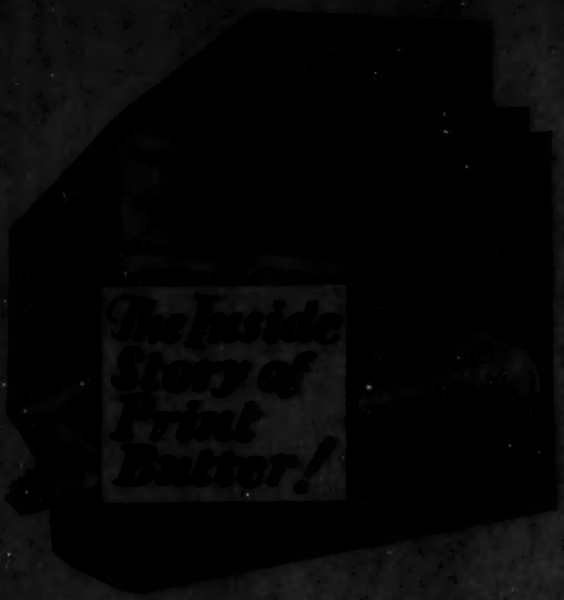

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